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Study of Klebsiella Species Producing ESBL Isolated from USG Probes

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Abstract

Ultrasonography machines are ideal vectors for cross infections. A busy machine may be used to scan many patients a day. The infections can be transmitted via ultrasound probes & coupling gel. Klebsiella species are frequent cause of infection in both community & Hospital. Klebsiella isolated from US probe is an important nosocomial pathogen & infections due to it are difficult to manage due to resistance to multiple antibiotics. So this study aimed to determine the percentage ESBL producing Klebsiella species isolated from US probes & to determine the antibiotic sensitivity pattern.

Keywords: Ultrasonography; US Probes; ESBL.

Introduction

Diagnostic equipment can be found every day in patient care centres in emergency rooms & impatient rooms & ICUs. Ultrasound is most commonly used diagnostic equipment. It has been used to image the human body over half a century [1]. However infections caused by diagnostic equipment as a result of contamination markedly threaten the health of patients especially of ICU. Surveillance studies have shown that contaminated medical devices act as source of infection [2].

Nosocomial infections have become an increasingly recognise problem with Ultrasonography probes can be one of the vehicles for spread of infections [3]. The finite studies are conducted in the field of possibility of hospital infection transmission by US probes and coupling gel [4,5].

With the increase in number of post operative and Immuno compromised patients being scanned effective guidelines for prevention of infection and Disinfection are necessary [4]. Literature shows limited studies regarding infection in post operative patients following ultrasonography [6,7].

Klebsiella pnemoniae resistant to Ceftazidime was isolated from US probes and Coupling gel in emergency rooms. A number of organisms such as Klebsiellia Acinetobactor baumannii, Enterococcus, Staphylococcus aureus have been isolated from gel 4. Gel and the probes have been traced to be a source of infection [6-10]. Present study is therefore carried out to determine the presence of the ESBL producing Klebsiella contamination on the US probes and also to determine the Antibiotics sensitivity pattern of isolates.

Materials & Methods

Prospective observational study was carried out in department of Microbiology, PDVVPF's Medical

College, & Hospital, Ahmednagar from Aug 2015 to Dec 2015. Total 220 swabs were taken randomly from from unclean US Probes of patients attending the radodiagnostic department. After ultrasound was carried out specimens were send to Microbiology Laboratory. Grams Stain was done, followed by culture on Blood agar and Mac Conkey agar at 37ºC for 24 Hrs. All strains were isolated as Klebsiella species by colony morphology and biochemical tests. (Indole, MR, Urease, Citrate, Glucose manitol, lactose, sucrose TSI, Catalase.) [11]. Total 25 Klebsiella which were isolated from Unclean Ultrasound probes out of 220 specimens were screened for ESBL production by using cefatazidime disc and those positive for screening test were subjected to confirmatory Phenotypic test for ESBL production as per CLSI gridlines. Out of 35 isolates of Klebsiella 20 were controls as ESBL producer while 15 were non ESBL producer. Antibiogram of the isolates was done by Kirby bauers disc diffusion method using antibiotic disc of Himedia.

All the klebsiella isolates having zone size less than < 22 m for ceftazidime were selected as suspicious for ESBL production by CLSI guidelines and these potential ESBL producing strains were further tested by phenotypic confirmatory test [12]. (CLSI performance standard.)

Phenotypic confirmatory test -Laun culture of the test isolates were done on MH agar. Antibiotic used were Ceftazidime(30 mcg) and combination of ceftazidime / Calvulanic acid discs have placed opposite to each other in MH agar and incubated overnight at 37ºC. Next day zone of inhibition around ceftazidime and Calvulanic acid was measured. Zone of inhibition around ceftazidime / Calvulanic acid is increased more than 5mm than that of ceftazidime alone. It is confirmed as ESBL Producer.

Klebsiella Pneumoniae ATCC 700603 was used as ESBL positive Control & E coli ATCC 25922 were used as ESBL negative control.

Results

Out of 220 specimens taken from unclean Ultrasound probes. 35 Klebsiella isolated from unclean Ultrasound probes. Amongst their 210 were isolated as control ESBL producer by phenotypic method.

Table 1: Percentage of Klebsiella Pneumoniae isolated from specimen

Total Specimens	Klebsiella Pneumoniae	Other	No Growth
220	35(15.9%)	55	130

Table 2: Percentage of ESBL Producing Klebsiella by screening test

Total Klebsiella Pneumoniae Isolated	ESBL by screening method
35	25

Table 3: Percentage of ESBL producing by phenotypic confirmatory test

Total Positive by ESBL Screning test	ESBL positive by phenotypic confirmatory test
25	18(51.4%)

Table 4: Antibiogram of ESBL producers

Antibiotic Name	Sensitive	Resistant
Ciprofloxacin	55.5%	44.4%
Amikacin	0%	100%
Cotrimoxazole	16.6%	83.3%
Amoxacilin / Clavulanic acid	33.3%	66.6%
Piperacillin / tazobactum	27.7%	72.2%
Imipenem	94.4 %	5.5%
Cefpodoxime	16.6%	83.3%
Ampicilin /Sullbactum	33.3%	66.6%
Colistin	100%	0%
Polymyxin B	100%	0%

Discussion

Present study detected 35 Klebsiella pneumoniae from 220 specimens from unclean ultrasound probes. Only 35 klebsiella isolates, 25 were ESBL producers by screening test. Total ESBL producing Klebsiella Pneumoniae were 18 out of 25 by phenotypic confirmatory test.

Nosocomial outbreaks due to ESBL producing Enterobactericeae have been described for ICU, Nursing homes, Obst. & Gynac wards [13]. In a study conducted by Oliver Gaillot [6] on 8 ESBL klebsiella were isolated from contaminated Ultrasound coupling gel. The common factor in their study, the hospital history of adult patients was USG performed on arrival in emergency room. Risk of contamination in Obstetric USG was recently emphasised by storment et al [14]. Because of the unusual mode of transmission of infection control measures such as isolation of colonised patients, use of gloves and hand washing did not halt the outbreak in the study. ESBL producing strains were resistant to variety of commonly used antimicrobials. Guntal et al [15] Akata F. et al [16]. Reported high prevalence of ESBL producing Klebsiella (19and 24).

The methods using US probes cleaned dry & neat cloth after each process as a standard method for probe decontamination but similar to other studies Ultrasound probes cleaned with dry & neat cloth could be a source of potential hospital infection. The use of 70% alcohol is used to clean the probes but its use is not recommended because it's shortens the life of probes. Muradeli et al [10] concluded that single paper probe cleaning was effective as immersion in chlorhexidine which reduce the bacterial contamination. In our study all ESBL producing isolates were 100% sensitive to Colistin and Polymyxin B followed by Imipenum 94.4%. All the strains were resistant to Amickacin.

Spencer and Spencer et al [17] also concluded that alcohol wipe can reduce the transmission of bacteria from ultrasound probes. Similar recommendations were given by Yasmin et al [18].

Conclusion

It has been found that ESBL producing klebsiella isolated from ultrasound probes is an important nosocomial pathogen & infection due to it can be hazardous. ESBL producing Klebsiella can be transmitted by Ultrasound probes and coupling gel. It is highly recommended that ultrasound departments must have their probe cleaning & sterilization procedures to assess whether they are safe in particular environment. And practitioners should ensure that risk of cross infection should minimize.

References

1. Edler, L. Lind storm K. The history of Echocardiography. Ultrasound Med. Biol. 2004;30:1565-1644.
2. Guinto CH, Bottone EJ, Raffalli JT, Montecalvo MA, Wormser GPEvaluation of dedicated stethoscopes as a potential source of nosocomial pathogens. AJIC Brief Rep. 2002;30(8):499-502.
3. Coella R etal. The cost of infection in surgical patients – A case study. J Hosp. Infec. 1993;25:239-250.
4. Matter EH, Hammad LF, Ahmad S, Elkersh T A. An investigation of bacterial contamination of ultrasound equipments' of university hospital in Saudi Arabia JCDR 2010;4;2685-90.
5. Hutchinison J, Runge W, Malrey M etal. Burkholdria cepacia infections associated with intrinsically contaminated ultrasound gel. The role of microbial degradation of parabens. Inf control Hosp Epidemio 2004;25:291-6.
6. Galliot O, Marrve Jouls C, Abachin E etal. Nosocomial outbreak of Klebsiella Pneumoniae producing SHV-s extended spectrum B lactamase, originating from a contaminated USG coupling gel. J Clinc. Microbiology may 1998;36(5):1357-60.
7. Ruthie kartaginer, Adinopupko, Charie Tepler. Do sonographers practice proper infection control techniques? J of Diagnostic Med. Sonogr.1997;13: 282-7.
8. Schabruns, Chipcase L, Richard H. Are therapeutic Ultrasound units a potential vector for nosocomial infections? Physiotherapy Res Int. June 2006;2:61-71.
9. Fowler C, MC Crackem D. Ultrasound probes: Risk of Cross infection & ways to reduce it. Comparison of clinical methods. Radiology 1999 Oct;213(1): 299-300.
10. Muradali D Gold W L, Philips A, Wilsons. Can Ultra sound probes and coupling gel be a source of Nosocomial infections in patients undergoing sonography ? An in vivo and invitro study. AJRAMJ Roentgen June 1995;164(6):1521 - 4.
11. Mackie and McCartney. Test book of Practical Medical Microbiology. 14Th edition Elsevier publications 2006; 131-150.
12. Clinical Laboratory standard institute (CLSI) performance, Standard for antimicrobial susceptibility testing 21st informational supplement, 2011;31(1).
13. Qunin J. P. Clinical Significance of ESBL Eur. J. Clinical Microbiology, Infectious diseases 1994;13(Suppl.): 39-42.

14. Storment J M. Monga M, Blanco J. D. In effectiveness of latest condoms in preventing contaminations of the transvaginal Ultrasound transducer head. South Med. J. 1997;90:206-208.

15. Gupta V. Etal- An update on newer B lactamases Indian Journal of Medical Research 2007;126(5):417-427.

16. Akata Fetal – Jain A, Mondal R. TEM SHV genes in extended spectrum B Lactamase producing Klebsiella species and their antimicrobial resistance pattern. Indian Journal Medical research 2008 Dec;128(6): 759-64.

17. Spencer & Spencer R. C. Ultrasound scanning of post operative wounds risk of cross infections Clin. Radio 1988;39:245-6.

18. Yasmin MK, Karadeniz M D, Dilek K, Simay KA, Deniz A, Sefika, Evaluation of the role of ultrasound Machine as a source of nosocomial and Cross infections. Investigative radiology; 2001;36(9);554-559.

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Identification of Coagulase Negative Staphylococci and Their Antibiogram Isolated from Various Clinical Specimen

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Abstract

Introduction: Coagulase negative staphylococci (CoNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections. More than 30 species of CoNS are recognized but only a few are commonly incriminated in human infections. CoNS species identification, which is still difficult for most clinical laboratories, is necessary in order to establish epidemiological trends, confirm treatment failures and to determine the cause of specific infections. Recently use of broad-spectrum antibiotics for treatment of CoNS leads to increase in the development of antibiotic resistance. Therefore the present study was conducted to identify frequency of CoNS species isolated from various clinical specimens and determination of their antimicrobial susceptibility pattern. *Material and Method:* Prospective study included a total of 238 non duplicate coagulase negative staphylococci from various clinical specimens. The specimens were collected using strict aseptic precautions and transported immediately to the laboratory. All the specimens received were processed further for identification by standard microbiological procedures [17]. Qualitative data were compared with the Chi-Square or Z test as appropriate. *Results:* Predominant species isolated in our study was *S. epidermidis* (42.02%) followed by *S. haemolyticus* (21.43%) and *S. saprophyticus* (15.13%). Majority of CoNS species were isolated from pus sample with the exception of *S. saprophyticus* which was more commonly isolated from urine. Majority of CoNS isolates were resistant to penicillin followed trimethoprim/ sulfamethaxazole and tetracycline and all isolates were sensitive to vancomycin, teicoplanin and linezolid. In our study, 58.82% species of CoNS were methicillin resistant. Antimicrobial resistance to most of the commonly used antibiotics was more among methicillin resistant CoNS as compared to methicillin sensitive and the difference was statistically significant. *Conclusion:* The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them warrants the need to identify various species of CoNS and determine their antibiotic resistance pattern.

Keywords: CoNS; *S. Epidermidis; S. Saprophyticus;* MRCoNS.

Introduction

Coagulase negative staphylococci (CoNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections [1]. They have been previously dismissed as contaminants are now emerging as important potential pathogens with the increase in number of severely debilitated patients and increased use of implants in hospitals. More than 30 species of CoNS are recognized but only a few are commonly incriminated in human infections [2]. *S. epidermidis* is the species most frequently isolated from human infections. CoNS species identification, which is still difficult for most clinical laboratories, is necessary in order to establish epidemiological trends, confirm treatment failures and to determine the cause of specific infections [3].

Recently use of broad-spectrum antibiotics for treatment of CoNS leads to increase in the development of antibiotic resistance [4]. Methicillin resistance among CoNS is particularly important due to cross resistance to all other beta-lactam agents and agents of other anti-microbial classes like macrolides and fluoroquinolones [3].

The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them warrants the need to identify various species of CoNS and determine their antibiotic resistance pattern. Therefore the present study was conducted to identify frequency of CoNS species isolated from various clinical specimens and determination of their antimicrobial susceptibility pattern.

Aims and Objectives

1. To identify Coagulase negative staphylococci (CoNS) from various clinical specimens.
2. To study the species distribution of CoNS.
3. To study antimicrobial susceptibility pattern of CoNS.

Material and Methods

The present study was carried out in the department of Microbiology, at a tertiary care hospital. Prospective study included a total of 238 non duplicate coagulase negative staphylococci from various clinical specimens during period of Nov 2013 to Oct 2015. Ethical clearance from the institutional ethical committee and animal ethical Committee was obtained.

A history was taken with reference to name, age and sex. Clinical history was recorded on a pre designed proforma. The specimens were collected using strict aseptic precautions and transported immediately to the laboratory. All the specimens received were processed further for identification by standard microbiological procedures [5].

The specimens were inoculated onto nutrient agar, blood agar and MacConkey agar plates. All plates were incubated aerobically at 37^0C and observed for growth after 18-24 hours of incubation.

Isolates of staphylococci were identified on the basis of colony characteristics on nutrient agar and blood agar. Fermentative, catalase positive and bacitracin resistant cluster forming Gram positive cocci were further tested by performing coagulase test (slide and tube test) [5]. Isolates which were negative by slide or tube method were labeled as coagulase negative staphylococci and further identified to species level by battery of biochemical tests.

Antimicrobial susceptibility of all the isolates was done on Muller Hinton agar (MHA) plate by using Kirby Bauer's disc diffusion method according to CLSI guidelines [6]. All the antibiotic discs were procured commercially from Hi- Media Laboratories Pvt. Ltd, Mumbai. Erythromycin and clindamycin were not tested for urinary isolates and nitrofurantoin was tested only for urinary isolates. Methicillin resistance was detected by using cefoxitin disc. Vancomycin resistance was detected by vancomycin screen agar method.

Data were presented as proportions and percentages, unless otherwise stated. Qualitative data were compared with the Chi-Square or Z test (Standard error of difference between two proportions), as appropriate. Probability (p) less than 0.05 was considered as significant.

Result and Observations

A total of 238 isolates of coagulase negative staphylococci were obtained from various clinical specimens amongst whom males were more (64.29%) as compared to females (35.71%).

Infection rate was more common in age group of 46-60 years followed by 31-45 yrs. Minimum rate was found in patients below 15 years of age group. Majority of isolates were obtained from patients admitted in various wards (65.97%) than the patients who were attending outpatient department (34.03%). CoNS isolates were more commonly obtained in patients from Surgery department followed by

Orthopedics and Obstetrics and Gynecology department. Maximum number of CoNS were isolated from pus (44.12%) followed by urine (27.73%).

Predominant species isolated in our study was *S. epidermidis* (42.02%) followed by *S. haemolyticus* (21.43%) and *S. saprophyticus* (15.13 %). *S. cohnii* (0.84) was least commonly isolated species. Majority of CoNS species were isolated from pus sample with the exception of *S. saprophyticus* which was more commonly isolated from urine.

Majority of CoNS isolates were resistant to penicillin followed trimethoprim / sulfamethaxazole and tetracycline and all isolates were sensitive to vancomycin, teicoplanin and linezolid. In our study, 58.82% species of CoNS were methicillin resistant. *S. cohnii* was isolated from two samples and both of them were resistant to methicillin. *S. haemolyticus* showed 62.75% methicillin resistance while *S. xylosus* showed only 28.57% methicillin resistance.

Antimicrobial resistance to most of the commonly used antibiotics was more among methicillin resistant CoNS as compared to methicillin sensitive and the difference was statistically significant, while resistance to gentamicin, amikacin, moxifloxacin and nitrofurantoin was somewhat higher among MRCoNS than MSCoNS and the difference was not statistically significant.

Table 1: Age wise distribution of patients with CoNS infection

Age Group (in years)	No. of Cases	Percentage (%)
< 15 years	21	08.82
16-30	52	21.85
31-45	60	25.21
46-60	78	32.77
>60	27	11.35
Total	**238**	**100**

Table 2: Department wise distribution of patients with CoNS infection

Department	No. of Cases	Percentage (%)
Surgery	75	31.51
Orthopedics	47	19.75
Obstetrics- Gynecology	37	15.55
ICU	25	10.51
Paediatrics	21	08.82
Medicine	18	07.56
ENT	15	06.30
Total	**238**	**100.00**

Table 3: Sample wise distribution of CoNS isolates

Type of Specimen	No. of Samples	Percentage (%)
Pus	105	44.12
Urine	66	27.73
Blood	24	10.09
Foley's catheter tip	12	05.04
Endotracheal Aspirate	10	04.20
Body fluids*	09	03.78
Central Line	08	03.36
Sputum	04	01.68
Total	**238**	**100**

Table 4: Species distribution of CoNS isolates

Species of CoNS	Percentage%
S. epidermidis	100(42.02)
S. haemolyticus	51(21.43)
S. saprophyticus	36(15.13)
S. hominis	15(6.30)
S. lugdunensis	14(05.88)
S. capitis	08(3.36)
S. xylosus	07(2.94)
S. warneri	05(2.10)
S .cohnii	02(0.84)
Total	**238**

Table 5: Antibiogram of CoNS isolates

Antibiotics	CoNS isolates (N=238)	
	Sensitive	Resistant
Penicillin G	59 (24.79%)	179 (75.21%)
Cefoxitin	98 (41.18%)	140 (58.82%)
Gentamicin	172 (72.27%)	66 (27.73%)
Amikacin	194 (81.51%)	44 (18.49%)
Erythromycin	101 (63.12%)	59 (36.88%)
Clindamycin	117 (73.12%)	43 (26.88%)
Ciprofloxacin	99 (41.60%)	139 (58.40%)
Moxifloxacin	190 (79.83%)	48 (20.17%)
Nitrofurantoin	60 (76.92%)	18 (23.08%)
Tetracycline	85 (35.71%)	153 (64.29%)
Trimethoprim / Sulfamethoxazole	83 (34.87%)	155 (65.13%)
Teicoplanin	238 (100%)	00 (00%)
Linezolid	238 (100%)	00 (00%)
Vancomycin	238 (100%)	00 (00%)

Erythromycin and clindamycin were tested against isolates from samples other than urine (N=160) and nitrofurantoin was tested only against urinary isolates (N=78)

Table 6: Methicillin resistance among CoNS species

Species	No. of MRCoNS (%)	No. of MSCoNS (%)
S. epidermidis (100)	60 (60%)	40 (40%)
S. haemolyticus (51)	32 (62.75%)	19 (37.25%)
S. hominis (36)	21 (58.33%)	15 (41.67%)
S. saprophyticus (15)	08 (53.33%)	07 (46.67%)
S. lugdunensis (14)	08 (57.14%)	06 (42.86%)
S. capitis (08)	04 (50 %)	04 (50%)
S. xylosus (07)	02 (28.57%)	05 (71.43%)
S. warneri (05)	03 (60%)	02 (40%)
S. cohnii (02)	02 (100%)	00 (00%)
Total (238)	**140 (58.82%)**	**98 (41.18%)**

Table 7: Antimicrobial resistance pattern among methicillin resistant and methicillin sensitive CoNS

Antibiotics	MRCoNS (N=140)	MSCoNS (N=98)	Total (N=238)	Z value	P value
Penicillin	140 (100)	39 (39.80)	179 (75.21)	12.35	<0.05
Gentamicin	41 (29.29)	25 (25.51)	66 (27.73)	0.65	>0.05
Amikacin	27 (19.29)	17 (17.35)	44 (18.49)	0.38	>0.05
Erythromycin	46 (47.42)	13 (20.63)	59 (36.88)	4.56	<0.05
Clindamycin	35 (36.08)	8 (12.70)	43 (26.88)	4.44	<0.05
Ciprofloxacin	94 (67.14)	45 (45.92)	139 (58.40)	3.31	<0.05
Moxifloxacin	30 (21.43)	18 (18.37)	48 (20.17)	0.59	>0.05
Nitrofurantoin	11 (25.58)	7 (20.00)	18 (23.08)	1.02	>0.05
Tetracycline	98 (70.00)	55 (56.12)	153 (64.29)	2.19	<0.05
Trimethoprim / sulfamethoxazole	102 (72.86)	53 (54.08)	155 (65.13)	2.99	<0.05

Figures in parentheses show parentages

Discussion

CoNS are increasingly being incriminated as a significant pathogen associated with healthcare infections and therefore, there is a need for identification of CoNS up to species level as has been emphasized by many investigators. The species identification is important in monitoring the reservoir and distribution of CoNS involved in healthcare-associated infections and will help to understand the pathogenic potential of individual CoNS species [7]. CoNS are a major cause of nosocomial bacteremia and septicemia, especially for the patients who have immune deficiency and malignancy, which can lead to morbidity and even mortality. Despite the recent introduction of antimicrobial agents and medical improvements in controlling the frequency and morbidity of staphylococcal infections, they are persistent as an important hospital and community pathogen [8].

In our study, a total of 238 isolates of coagulase negative staphylococci were obtained from various clinical samples.

Age and sex wise distribution of patients in the present study has followed natural epidemiological patterns. Male to female ratio among the included cases was 1.8:1. Exactly similar ratio was shown by Ravindran et al [9] (2014) while Ahmad et al [4] (2012), and S A Sardar et al [3] (2015) documented male to female ratio as 1.3:1 and 1.2:1 respectively.

In the present study, out of 238 patients, majority belonged to age group 46-60 years (32.77%) followed by 31-45 years (25.21%). S A Sardar et al [3] (2015) and Usha et al [1] (2015) has found the most affected age group in their study as 31 to 50 years and 31 to 40 years respectively. Surekha et al [10] (2011) has observed the most affected age group in her study was >40 years (39.05%).

In the present study majority of CoNS isolates were obtained from patients admitted in various wards (65.97%) than OPD patients (34.03%). Similarly Muhammad et al [11] (2013) reported that isolation rate of CoNS was higher from inpatient department (69%) than from patients attending OPDs (31%). Many workers have expressed their view that the duration of hospital stay is directly proportional to the higher prevalence of the infection since the rate of isolation of organisms is higher in indoor than outdoor patients. This could be attributed to infection in wards from patient to patient via hands of nursing staff. Thus frequent hand washing to prevent spread of organisms should be encouraged.

In the current study, rate of isolation of CoNS was higher from surgery department (31.51%) followed by orthopedic (19.75%) and OBGY department (15.55%). Similar observation was made by Tanusri Biswas et al [12] (2008) who showed more isolation from surgery department (59.67%) followed by orthopedic department (19.35%). While in a study done by F Koksal et al [8] (2006), maximum isolation was from intensive care unit (30%), followed by surgery department (17.50 %).

In the present study, maximum number of CoNS were isolated from pus (44.12%) followed by urine samples (27.73%). This finding is comparable with study done by R Goyal et al [13] (2006), who obtained 38.2% of CoNS isolates from pus followed by urine samples (28.4%). Surekha et al [10] (2011), in her study reported 33.3 % of CoNS isolates were from pus followed by urine samples (27.1%) while Usha et al [1] (2015) isolated CoNS species more frequently in blood (52%) than pus (32 %). U Mohan et al [2] (2002) showed maximum isolation of CoNS from urine (48.43%), followed by pus (17.70%).

In our study, as many as nine CoNS species were isolated with varying frequencies from different clinical specimens. Amongst which *S. epidermidis* was the most predominant isolate (42.02%) followed by *S .haemolyticus* (21.43%) and *S. saprophyticus* (15.13%). This finding very well correlates with various studies like F. Koksal et al [8] (2009), Ahmad et al [4] (2012) and Mohammad Mubashir et al [7] (2014) who also reported *S. epidermidis* as most frequently encountered clinical isolates followed by *S. haemolyticus.* while Surekha et al [10](2011), Shubhra singh et al [14] (2008), U Mohan et al [2] (2002) and KS Seetha et al [15] (2000) obtained *S. epidermidis* as the most frequent isolate followed by *S. saprophyticus* and *S. haemolyticus*.

In our study, the specimens from which the CoNS species were isolated showed propensity of certain species for specific culture type. *S epidermidis* which was the predominant isolate was most commonly isolated from pus (49%). Similarly Shrikande et al [16] (1996) had shown maximum isolation of *S. epidermidis* from pus (38.46%), while Usha et al [1] (2015) reported maximum isolation of *S. epidermidis* from blood (59.37%) and Ahmad et al [4] (2012) reported maximum isolation from urine (51.5%). A total of 69.44% isolates of *S. saprophyticus* were recovered from urine where its pathogenic potential is well documented. S A Sardar et al [3] (2015) also reported that *S. saprophyticus* was predominantly isolated from urine (75%).

In the present study, antibiotic susceptibility testing showed maximum resistance to penicillin (75.21%). Somewhat similar penicillin resistance was reported by Shrikande et al [16] (1996) (63.10%). Mohammad et al [7] (2014) and Surekha et al [10] (2014) exhibited higher penicillin resistance of 99.5% and 94.79% respectively in their study. In our study, trimethoprim / sulfamethoxazole resistance was 65.13% which correlates with the findings by S A Sardar et al [3] (2015) and Mayhall CG et al [17] (2004) who showed 59% and 76% resistance to same antibiotic respectively. Koksal et al [8] (2009) reported lower resistance of 47%. Resistance for tetracycline was 64.29% in our study which was similar to the resistance shown by A. Sheikh et al [4] (2012) (66%) while E. Akinkumni et al [18] (2010) showed only 34% resistance.

In the present study, lower gentamicin resistance was found (27.73%) which was corresponding to the results shown by Ravindran et al [19](2014) (20%). S A Sardar et al [3] (2015) and Mohammad Mubashir et al [11] (2014) reported higher gentamicin resistance of 53% and 82% respectively. While Akinkumni et al [18] (2010) reported lower resistance as compared to our study (8%).

All the isolates in the present study were sensitive to vancomycin, teicoplanin and linezolid. Similar findings were shown by S A Sardar et al [3](2015), Ravindran et al [19] (2014) and Koksal et al [8] (2009) hence it may be prudent to use these agents for empirical treatment of serious infections in hospitalized patients [18]. But Manikandan et al [20] (2005) in his study reported vancomycin resistance of 65.7%. Vancomycin has long been considered as an antibiotic of last resort but extensive use of glycopeptide in hospital has been related to decreased susceptibility to these agents. In comparison to our study, Samant et al [21] (2012) reported 4.1% and 0.6% resistance to linezolid and Teicoplanin respectively

In our study, 58.82% CoNS isolates were methicillin resistant by cefoxitin disc diffusion method. Similar to our study, Shubhra et al [14] (2008) and F. Koksal et al [8] (2009) showed 62% and 67.5% MRCoNS respectively in their study. Higher prevalence rate of 78.84% of MRCoNS was given by Artee et al [22] (2015). In our study, maximum resistance to methicillin was shown by *S .haemolyticus* (62.75%) while Shubhra et al [14] (2008) and Surekha et al [10] (2011) showed higher methicillin resistance of 76% and 90% in *S. haemolyticus* respectively. In our study, *S. epidermidis* also showed significant percentage of resistance to methicillin (60%). Similar finding were shown by Surekha et al [10] (2011) and Kumari et al [23] (2001) who showed methicillin resistance of 66% and 65% in *S. epidermidis* respectively. Lowest methicillin resistance was shown by *S. xylosus* in our study.

This increasing prevalence and isolation rate of MRCoNS is alarming because of its self-involvement in the diseased condition and possibility of transferring the mecA gene to *S. aureus*. Therefore continuous monitoring, strict antibiotics policies and resistance program is mandatory, which will contribute in implementation of infectious measures [22].

In the present study, antimicrobial resistance to most of the commonly used antibiotics was more among methicillin resistant CoNS as compared to methicillin sensitive and the difference was statistically significant. Similar results were obtained by Akinkumni et al [18] (2010) who showed that there was reduced susceptibility to most of the antibiotics by MRCoNS as compared to MSCoNS isolates.

Summary and Conclusion

CoNS are a major cause of nosocomial bacteremia and septicemia, especially for the patients who have immune deficiency and malignancy, which can lead to morbidity and even mortality. Despite the recent introduction of antimicrobial agents and medical improvements in controlling the frequency and morbidity of staphylococcal infections, they are persistent as an important hospital and community pathogen. The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them warrants the need to identify various species of CoNS and determine their antibiotic resistance pattern.

References

1. Usha M, Shwetha D, Vishwanath G. Speciation of coagulase negative Staphylococcal isolates from clinically significant specimens and their antibiogram. Indian J Pathol Microbiol [Internet]. 2013;56(3):258. Available from: http://www.ijpmonline.org/text.asp?2013/56/3/258/120383.
2. U Mohan, N Jindal PA. 11/16/2014 Species distribution and antibiotic sensitivity pattern of Coagulase negative staphylococci isolated from various clinical specimens: U Mohan, N. Indian J Med Microbiol. 2014;20(1):45–7.
3. Sardar S, Singh M, Basireddy S AS. Coagulase negative staphylococci among clinical isolates in a tertiary care centre. Int J pharma bio-sciences. 2015;6(1):229–36.
4. Sheikh AF, Mehdinejad M. Identification and determination of Coagulase negative staphylococci species and antimicrobial susceptibility pattern of isolates from clinical specimens. African J Microbiol Res. 2012;6(8):1669–74.
5. Washington Winn Jr, Stephen Allen, William Janda, Elmer Koneman, Gary Procop PS. Identification of Coagulase negative staphylococci. Konemans color Atlas and Textbook of Diagnostic Microbiology. 6th ed. lippincott williams and wilkins; 2006.p.649–62.
6. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty Second Informational Supplement. CLSI Document M100-S22,Clinical and Laboratory Standard Institute,950 West Valley Road,Suite 2500,Wayne,Wayne PA19087 USA2014.
7. Mubashir M, Khan A, Faiz A, Ashshi AM. Clinically significant Coagulase negative staphylococci and their antibiotic resistance pattern in a tertiary care hospital. jpma. 2013;64(10):1171–4.
8. Koksal F, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. Microbiol Res [Internet]. Elsevier; 2009;164(4):404–10. Available from: http://dx.doi.org/10.1016/j.micres.2007.03.004.
9. Shobana CS. Virulence, Speciation and Antibiotic Susceptibility of Ocular Coagualase Negative Staphylococci (CoNS). J Clin Diagnostic Res [Internet].

2014;8(5):33–7. Available from: http://jcdr.net/article_fulltext.asp?issn=0973-709x&year=2014&volume=8&issue=5&page=DC33&issn=0973-709x&id=4395.

10. Asangi SY, Mariraj J, Sathyanarayan MS. Speciation of clinically significant Coagulase negative staphylococci and antibiotic resistant patterns in a tertiary care hospital. Int J Biol Med Resrarch. 2011;2(3):735–9.
11. Muhammad Murad Ehsan, Zahida memon, M.Owasis GF. Identification and intibiotic seneitivity pattern of Coagulase negative staphylococci in various clinical specimens. Pak J Med Sci. 2013;29(06).
12. Tanusri Biswas, Munmun Das (Sarkar), Hirak Jyoti Raj, Raston Mondal, C. Srilatha. Species Identification of Staphylococci from PUS Samples Using Commercial identification system and their antimicrobial susceptibility pattern with special referenceto methicillin. JK-Practitioner. 2013;18(3-4):21–30.
13. R Goyal, NP Singh, A Kumar, I Kaur, M Singh, N Sunita MM. Simple and economical method for speciation and resistotyping of clinically significant coagulse negative staphylococci. Indian J Med Microbiol. 2006;24(3):201–4.
14. Shubhra singh,Gopa Banerjee, S K Agarwal, Mala Kumar RKS. Simple method for speciation of clinically significant coagulase Staphylococci and its antibiotic sensitivity pattern in NICU of tertiary care centre. 2008;19(2):05–8.
15. KS Seetha, PK Santosh PS. Study of Coagulase negative staphylococci Isolated from Blood and CSF Cultures. Indian J Pathol Microbiol. 2000;43(1):41–5.
16. Shrikhande S, Thakar YS,Pathak AA SA. Species distribution of clinical isolates Staphylococci. Indian J patho / Microbiol. 1996;39(3):207–10.
17. CG M. Hospital epidemiology and infection control 3rd ed. Philadelphia: Lippincott wiliam and Wilkins. 2004.p 495–510.
18. Akinkunmi EO, Lamikanra A. Species Distribution and Antibiotic Resistance in Coagulase negative staphylococci Colonizing the Gastrointestinal Tract of Children in Ile-Ife, Nigeria. Trop J Pharm Res. 2010;9(1):35–43.
19. Ravindran Priya, Arumugam Mythili, Yendremban Randhir Babu Singh HS. Virulence, Speciation and Antibiotic Suceptibility of Ocular Coagulase negative staphylococci. J Clin diagnostic Res. 2014;8(5):33–7.
20. Manikandan P, Bhaskar M, Revathy R, John RK, Narendran K, Narendran V. Speciation of Coagulase Negative Staphylococcus causing Bacterial Keratitis. Indian J Ophthalmol. 2005 Mar;53(1):59-60.
21. Samant Sharvari, A, Pai Chitra G. Evaluation of different detection methods of biofilm formation in clinical isolates of staphylococci. Int J Pharma Bio Sci. 2012;3(4):724–33.
22. Artee A, Tyagi DSST. Prevalance and antimicrobial susceptibility of methicillin resistance Staphylococci isolated in a tertiary care hospital of rural Gujrat, india. J Biomed Pharm Res. 2015;4(4):35–41.
23. Namrata Kumari, Amereshwar Rai, Jaiswal CP, Anim Axess SS. Coagulase negative staphylococci as casuative agents of urinary tract infections prevalence and resistsnce status in IGIMS, Patna. Indian J patho / Microbiol. 2001;44(4):413–9.

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Study of Bacteriological Profile of Ear Discharge in ASOM Cases at a Tertiary Care Hospital

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Abstract

Introduction: Acute otitis media(AOM) is a common condition with a high morbidity and low mortality. Acute suppurative otitis media (ASOM) is termed as the rapid and short onset of signs and symptoms of inflammation in the middle ear. It generally affects younger age group,with signs: otalgia, otorrhoea, fever, etc. bacterial profile of ASOM is clinically very important for accurate treatment. *Material & Methods:* Present study was carried in Department of Microbiology during the period September 2006 to August 2008 at Government College and Hospital, Ambajogai. A total of 179 clinically diagnosed cases of otitis media were included in study in the present study. A pretested proforma was filled in each case noting particulars in each case. Patients with ear discharge from acute otitis media with or without complications were selected, indoor as well as outdoor patients with discharging ears, had not received treatment for at least seven days prior to taking the samples. *Results:* Of the 179 swabs of ASOM, 162 swabs were culture positive (90.50%). Aerobes were isolated from 146 swabs (81.56%). Streptococcus pneumoniae was commonest isolate, 28 (18.79%). Anaerobes were isolated from 4 swabs (2.23%).Prevotella melaninogenica was predominant isolate, 2 (50%). *Conclusions*: ASOM is a common ENT condition with high morbidity and in our study a total of 179 cases were recorded with features of ASOM. More than 90% of samples were culture positive which was comparable to results by other authors. Aerobes were isolated in more than 80% of cases and Streptococcus pneumoniae was being comment organism.

Keywords: ASOM; Otitis Externa; Bacteriological Profile; Ear Discharge; Aerobes; Anaerobes.

Introduction

Discharge from the ear is one of the commonest symptoms of infections of the ear. Infection of the ear is categorized into otitis externa (infection of external ear) and otitis media (infection of middle ear), the most common cause being otitis media [1]. As external and middle ear are exposed to outer environment and nasopharynx respectively, these sites are likely to be infected when the natural milieu is disturbed. Otitis externa is a generalized condition of the skin of the external auditory canal that is characterized by edema and erythema associated with itchy discomfort and usually an ear discharge [2]. Otitis Media comprises of the inflammation of the middle ear cleft. It can be

acute, subacute or chronic. 3 Acute Suppurative Otitis Media (ASOM): It is the commonest ear pathology in otorhinolaryngological practice, also the commonest pediatric otorhinolaryngological presentation.[3,4]

Acute suppurative otitis media (ASOM) is termed as the rapid and short onset of signs and symptoms of inflammation in the middle ear. It generally affects younger age group,with signs: otalgia, otorrhoea, fever, etc. Common organisms responsible for it are: Streptococcus pneumoniae, Haemophilus influenzae, Branhamella catarrhalis, Streptococcus-Gp A, Staphylococcus aureus, etc [5].

Acute otitis media(AOM) is a common condition with a high morbidity and low mortality. In the United Kingdom, each year, about 30% of children aged under 3 years visit their general practitioner with AOM [6]. In United States, AOM accounts for 18% of ambulatory care visits per year among preschool children [7,8]. The overall prevalence of active and inactive chronic otitis media(COM) given by UK National Study is 4.1% [9].

Changing trends of drug sensitivity in recent years have been seen due to improved bacteriological techniques. Studies in 1980s of the bacteriology of acute otitis media (AOM), found that H. influenzae was the dominant pathogen representing 53% of the isolates(verses 19% for S. pneumoniae). In 1990s, studies documented a shift in the persistent AOM population to S. pneumoniae as the dominant pathogen,with penicillin nonsusceptible S. pneumoniae (PNSP) accounting for 31% to 70% of S. pneumoniae recovered from these cases [10].

Material Methods

Present study was carried in Department of Microbiology during the period September 2006 to August 2008 at Government College and Hospital, Ambajogai. A total of 179 clinically diagnosed cases of otitis media were included in study in the present study. A pretested pro-forma was filled in each case noting particulars in each case. Criteria for selection: 1) Patients with ear discharge from acute otitis media with or without complications were selected. 2) Indoor as well as outdoor patients with discharging ears were taken from the Department of E.N.T. 3) Patients had not received treatment for at least seven days prior to taking the samples.

Results

The Table 1 shows the prevalence of organisms in present study. Out of the 179 samples of ASOM, the aerobes are isolated from 146 samples (81.56%) and anaerobes from 4 samples(2.23%). No growth is seen in 19 samples . Of the 402 samples of CSOM, the total aerobic samples are 282 (70.15%) and anaerobic samples are 296 (73.63%). 3 samples show no growth.

The Table 2 shows the distribution of bacteria in ASOM: Aerobes are isolated from 146 samples (81.56%) of the total samples and anaerobes are isolated from 4 samples (2.23%). There are no samples showing mixed growth.

Table 1: Prevalence of aerobic and anaerobic microorganisms in ASOM

Distribution	ASOM
Samples	179
Total aerobes	146
Total anaerobes	4

Table 2: Prevalence of monomicrobial and polymicrobial organisms isolated in ASOM

Organisms	Nature	Samples	Strains
Aerobes	Monomicrobial	143	143
	Polymicrobial:2 isolates	3	6
	Total	146	149
Anaerobes	Monomicrobial	4	4
	Polymicrobial	-	-
	Total	4	4
	Total	**150**	**153**

Table 3: Distribution of aerobes isolated in ASOM
A. Monomicrobial isolates in ASOM:

Sr. No	Organisms Isolated	Isolates	% (N=143)
1.	Streptococcus pneumoniae	27	18.88%
2.	Staphylococcus aureus	24	16.78%
3.	Streptococcus pyogenes	17	11.89%
4.	Escherichia coli	12	8.39%
5.	Klebsiella species	12	8.39%
6.	Pseudomonas aeruginosa	12	8.39%
7.	Proteus vulgaris	11	7.69%
8.	Streptococcus viridians	10	6.99%
9.	Proteus mirabilis	9	6.29%
10.	Staphylococcus epidermidis	8	5.59%
11.	Diphtheroids	1	0.69%
	Total	143	

B. Aerobic polymicrobial organisms in ASOM : 2 organisms

Sr. No.	Organisms	Samples	Strains
1.	Streptococcus pyogenes +Staphylococcus aureus	2	4
2.	Streptococcus pneumoniae +Pseudomonas aeruginosa	1	2
	Total	3	6

Table 4: Anaerobes in ASOM

Sr. No.	Anaerobe	Samples	Strains
1.	Prevotella melaninogenica	2	2
2.	Peptostreptococcus magnus	1	1
3.	Propionibacterium acnes	1	1
	Total	**4**	**4**

Out of 146 samples of aerobes, 143 samples have isolated single organism 143(97.95%) strains and 3 samples have isolated 2 types of organisms, 6 (2.05%) strains. All the 4 anaerobic samples show single organism.

Of the 146 samples of aerobes, 143 samples are monomicrobial (97.94%). Commonest organism is Streptococcus pneumoniae, isolated in 18.88% of isolates. The polymicrobial organisms are recovered from 3 samples (2.05%) of the total aerobic samples. Streptococcus pyogenes +Staphylococcus aureus is isolated from 2 samples out of the 3 polymicrobial samples.

Anaerobes are isolated from 4 samples (2.23%) and they are all monomicrobial organisms.

Prevotella melaninogenica is the commonest, isolated in 50% of the anaerobic samples of ASOM, followed by Peptostreptococcus magnus, and Propionibacterium acnes, isolated in 1 sample each. The findings from above table suggests that anaerobic organisms though rare, are still present in acute cases of otitis media.

Discussion

Monomicrobial and polymicrobial distribution of aerobes and anaerobes in ASOM:

ASOM Aerobes: In ASOM, aerobes are present in 146 (81.56%) of the total samples and anaerobes in 4(2.23%)of the samples. There are no samples showing mixed growth. This is consistant with Celin et al [11] (82%), Zeilnik et al [12] (63.9%), Jokipii et al [13] (69.98%).

Polymicrobial Isolation in ASOM: Out of 146 samples of aerobes, 143 (97.94%) are monomicrobial and 3(2.05%) are polymicrobial containing 2 different organisms, this is in accordance with Shurin et al [14] (4.25%), Celin et al [11] (5.88%). Friedman (1957) [15] isolated polymicrobials in 11.7% cases which is higher than present study. Kikuta et al(2007) [16], in their study on ASOM cases predicted that lower age and presence of multiple bacteria.

ASOM Anaerobes: In present study, only 4 monomicrobial anaerobes are recovered (2.23%) and show no polymicrobials.

Anaerobes have a limited role in acute otitis media. Celin et al [11] (1991) isolated anaerobes in 5% .

Distribution of Organisms in ASOM

Aerobes: 149 strains of aerobes are isolated from 146 samples. Streptococcus pneumoniae is the most common organism found in 18.79% of isolates. Celin et al [11] (21%), Kilpi et al [17] (26%), Fairbanks et al [18] (25%), Zeilnik et al [12] (40.4%), present study is in accordance with above studies. S.pneumoniae is one of the commonest organism of ASOM.

Comparison of Commonest Organisms of ASOM

Author	S.pneumoniae	S.pyogenes	S.aureus
Friedman et al[15]	4.2%	27.4%	41.8%
Baruah et al[18]	2.91%	0.97%	33.98%
Shurin et al[14]	12.85%	2.85%	4.28%
Sriwardhana et al[20]	2.7%	0.4%	2.7%
Celin et al[11]	21%	3%	3%
Zeilnik et al[12]	40%	-	14%
Present study	18.79%	12.75%	17.45%

Comparison of Bacteria in Different Studies in ASOM

Organism	Friedman et al[15]	Baruah et al[19]	Shurin et al[14]	Present Study
Streptococcus viridans	27.4%	7.69%	1.43%	6.99%
Streptococcus pyogenes	6.43%	0.00%	2.85%	12.75%
E.coli	4.2%	0.00%	1.43%	8.39%
Pseudomonas aeruginosa	5.5%	15.38%	1.43%	8.72%
Klebsiella pneumoniae	0.00%	38.40%	1.43%	8.39%
Staphylococcus epidermidis	0.00%	23.08%	-	5.59%
Diphtheroids	0.00%	0.00%	-	0.69%

Comparison of Commonest Organisms of ASOM

Lower percentage of Streptococcus pneumoniae than ours was seen by Freidman et al [15] (1957), Baruah et al [19] (1972) and Sriwardhan et al [20] (1989).

Other organisms in present study are Pseudomonas aeruginosa (8.72%), E.coli (8.39%), Klebsiella species (8.39%), Proteus vulgaris (7.69%), Streptococcus viridians (6.99%), Proteus mirabilis (6.29%), Staphylococcus epidermidis (5.59%) and Diphtheroids(0.69%). Gram negative bacteria are responsible for 20% cases of otitis media in young infants [4].

Anaerobes: Out of the 179 swabs of ASOM, anaerobes are isolated in 2.23% of samples. Our study shows Prevotella melaninogenica 2 strains, Peptostreptococcus magnus and Propionibacterium acnes,1 strain each.

Brook (1987) [21] isolated anaerobes, mainly Prevotella melaninogenica and gram negative cocci. Celin(1991) [11] isolated 2 strains of Propionibacterium acnes in his study. Propionibacterium acnes could be considered as a component of the skin flora of the external auditory canal and is rarely a pathogen. Confirmation is done by reisolation of this organism from the same patient.

Conclusions

ASOM is a common ENT condition with high morbidity and in our study a total of 179 cases were recorded with features of ASOM. More than 90% of samples were culture positive which was comparable to results by other authors. Aerobes were isolated in more than 80% of cases and Streptococcus pneumoniae was being comment organism.

References

1. Arjyal C, Adhikari S, Shrestha J. Bacteriological study of ear discharge in Bir hospital. Journal of Nepal Medical Association 2002;41:318-22.
2. Carney AS. Otitis externa and otomycosis. In: Gleeson M, Browning GG, Burton MJ, Clarke R, Hibbert J, Jones NS, Lund VJ, Luxon LM, Watkinson JC, editors. Scott-Brown's Otorhinolaryngology, Head and Neck Surgery. 7th ed. Great Britain: Edward Arnold; 2008.p.3351-52.
3. Iseh KR, Adegbite T. Pattern and bacteriology of acute supportive otitis media in Sokoto, Nigeria. Annals of African Medicine 2004;3(4):164-66.
4. Kaur P, Sood AS, Sharma S, Aggarwal A. Bacteriological profile and antibiotic resistance

pattern of ear discharge in a tertiary care hospital. Indian J Microbiol Res 2016;3(4):423-428.

5. Berman S. Otitis media in children. N. Engl .J. Med. 1995;332:1560-1561.
6. Michael E. Pichichero. Evolving Shifts in Otitis Media Pathogens: Relevance to a Managed Care Organization. The American Journal Of Managed Care. 2005 Aug;11(6 Supp):S192-201.
7. Park MK,Jung MH, Kang HJ. The changes of MRSA infection in chronic suppurative otitis media. Head Neck Surg. 2008 Sep;139(3):395-8.
8. Rama Rao MV,Jayakar PA: Bacteriological Study of Chronic Suppurative Otitis Media. J. Indian M.A. 1980 July16;75(2):3.
9. Grundfast KK. Management of Otitis Media and the New Agency for Health Care Policy and Research guidelines. Arch Otolaryngol Head Neck Surg; 1974 Aug;120:794-798.
10. Shenoi PM. Scott Brown's Textbook of Otolaryngology, 1987, 5th edition; Vol 3, Booth J.B., Kerr A. G., eds. Butterworth, London: 1987;3:218.
11. Celin SE et al, Bacteriology of acute otitis media in adults, JAMA 1991;266(16):2249-52.
12. Zeilnik BJ, Anna Bielicka; Evaluation of antibiotic resistance in material isolated from middle ear in children with ASOM not responding to treatment; Otolaryngol Pol 2007;61(5):892-7.
13. Jokipii AMM., Karma P, Ojalak, Jokipii L. : Anaerobic bacteria in chronic otitis media; Arch Otolaryngol, 1977;103:278-280.
14. Shurin PA., Howie VM., Pelton SI. et al : Bacterial etiology of otitis media during the first 6 weeks of life. Jr. Pediatrics, 1978;92:893.
15. Friedman I : Discussion on recurrent otitis media in children. Proceedings of Royal Society of Medicine, 1957;50:406-409.
16. Kikuta S,Ushio M,Fujimaki Y,Kaga K. Factors associated with the presence of drug resistant bacteria and recurrent AOM in children. Acta Otolaryngol 2007 Dec;127 (Suppl 559):5-8.
17. Kilpi T, Herva E, Koijalainen T, Syrjanen R, Takala A.K,. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. National Pubic Health Institute, Helsinki, Finland. 2001 Jul;20(7):654-62.
18. Fairbanks,DNF. Pocket guide to antimicrobial therapy in otola. Lo. head and neck S.5th ed. American Academy of Otolaryngology-Head and Neck surgery Foundation, Washington. 1989;13:28-29.
19. Baruah PC, Agarwal SC, Arora MML. and Mehra Y N: Clinical and Microbiological studies in CSOM in Chandigarh. Ind. Jr. Otol 1972;24(4):157.
20. Sriwardhana KB, Howard AJ, Dunkin KT. Bacteriology of Otitis Media with effusion. The Journal of Otolaryngology and Otology March 1989;103:253-56.
21. Brook I, Paula Yocum, Kiran Shah. Aerobic and Anaerobic Bacteriology of Concurrent Chronic Otitis Media With Effusion and Chronic Sinusitis in Children. Arch Otolaryngol Head Neck Surg. 2000;126(2):174-176.

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Declining Trends in the Prevalence of Hepatitis B Virus Infection in Antenatal Women; Role of Vaccination

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Abstract

Context: Hepatitis B Virus infection is one of a serious public health problem worldwide and it is 50±100 times more contagious than HIV. Many of the carriers do not realize that they are infected with the virus rendering the HBV to be known as a "silent killer". The transmission of hepatitis B virus (HBV) is parenteral, sexual and perinatal. Prevention of vertical transmission of HBV is extremely important, because HBV infection in early life usually results in a chronic carrier state. *Aim:* To study the prevalence of Hepatitis B surface Antigen (HBsAg) positive among pregnant women referred to Department of Microbiology, Karnataka Institute of Medical Sciences during the study period. *Materials and Methods:* The study is a prospective study conducted by collecting blood samples from 3223 antenatal women with age ranging from 21-30 years, attending the department of Microbiology during the study period. Screening of HBs Ag was done by rapid immunochromatographic method. *Results:* Out of 3223 antenatal women screened 48 were positive accounting for 1.49%. In the age group of 21-25 years 34 (1.45%), 26-30 years 13 (1.92%) and 31-35 years one (0.5%) antenatal women were positive The highest prevalence was observed in the age group 26-30 years (1.92%). *Conclusion:* HBV during pregnancy is associated with a high risk of maternal complications, has a high rate of vertical transmission. The study suggests expansion of the Hepatitis B vaccination program to reduce the risk of HBV among pregnant women.

Keywords: Hepatitis B Virus; Antenatal Women; HBsAg; Universal Immunisation Programme.

Introduction

Hepatitis B virus (HBV) is a double-stranded DNA virus belonging to the *Hepadnaviridae* family. Hepatitis B is a potentially life-threatening liver disease caused by HBV. It is a major global health problem and the most serious type of viral hepatitis. It can cause chronic liver disease and put people at high risk of death from cirrhosis of liver and liver cancer [1].

Hepatitis B virus (HBV) infection is a serious public health problem worldwide [2]. Prevalence of Hepatitis B in pregnant women worldwide is 0.25 to 1.5% whereas in India it is 0.2 to 7.7% [3]. Before HBV vaccine was integrated into the routine immunization programme, in India in 2002 and was scaled up nationwide in 2011, about 10% to 30% babies were becoming HBV carriers amongst mothers who were HBsAg positive but HBeAg negative. However, perinatal infection was higher (70% to 90%), when

mothers were also HBeAg positive. The possible routes of transmission for HBV from infected mothers to infants are; transplacental-in utero, natal-during delivery [4].

Transmission of HBV from carrier mothers to babies can occur during perinatal period, and is important factor in determining the prevalence of infection in highly endemic areas [5]. The present study was designed to assess the prevalence of hepatitis B infection, among otherwise healthy pregnant females.

Materials and Methods

The present study is a prospective study. The study was conducted in the Department of Microbiology.

Five ml of blood sample was collected from all the pregnant women attending the OPD of Department of Microbiology with proper aseptic precautions. The serum was separated by centrifugation of 3000 rpm for 15 minutes. The centrifuged serum samples were screened for Hepatitis B surface Antigen (HBsAg) using rapid immuno-chromatographic kits from Qualpro diagnostics.

Results

A total of 3223 antenatal cases underwent screening for Hepatitis B surface Antigen during the study period. Out of which 48 were positive accounting for a seroprevalence of 1.49% (Table 1).

In the age group of 21-25 years 34 (1.45%), 26-30 years 13 (1.92%) and 31-35 years one (0.5%) antenatal women were positive The highest prevalence was observed in the age group 26-30 years (1.92%) as sown in Table 1.

Statistical Analysis

SPSS software version 2.0

Table 1: Age-wise seroprevalence of HBsAg among pregnant women.

Age Group (years)	No. of Antenatal cases underwent screening for HBsAg	No. of HBsAg positive antenatal cases	Percentage of HBsAg positive antenatal cases (in %)
21 – 25	2346	34	1.45
26 – 30	677	13	1.92
31 – 35	200	01	0.5
Total	3223	48	1.49

(HBsAg: Hepatitis B surface Antigen)

Table 2: Comparison of prevalence of HBsAg among antenatal women before and after the introduction of HBsAg Vaccine (2002)

Name of the study done before 2002	Prevalence of HBsAg among ANC in the study (%)	Name of the study done after 2002	Prevalence of HBsAg among ANC in the study (%)
Nayak NC et al[7] (1987)	3.7%	Chatterji S et al[13] (2009)	0.82%
Biswas SC et al[8] (1989)	2.3%	Pandy et al[14] (2011)	1.1%
Gupta I et al[9] (1992)	2.48%	Dwivedi M et al[5] (2011)	0.9%
Gill HH et al[10] (1995)	5%	Sibia et al[15] (2016)	1.11%
Mittal SK et al[11] (1996)	4.6%	Rajendiren S et al[16] (2017)	1.01%
Prakash C et al[12] (1998)	9.5%	Mishra S et al[3] (2017)	1.09%

(HBsAg: Hepatitis B surface Antigen)

Discussion

Hepatitis B virus is a DNA hepadnavirus. It is a 47-nm spherical virus that possesses several antigens of importance for diagnosis and pathogenesis. Several genotypes of hepatitis B virus have distinct clinical implications. Genotype B is found more commonly in infections that results in acute and chronic hepatitis and is a etiological agent of hepatocellular carcinoma.

Hepatitis B virus has a long incubation period (45-120 days) and is transmitted primarily by parenteral route. Other parenteral routes have included acupuncture and tattooing. This virus can be transmitted by sexual contact and has produced epidemic disease in male homosexuals, prostitutes, and abusers of intravenous drugs. Perinatal infection occurs, but breast milk does not appear to play a role in transmission [6]. Hepatitis B Virus causes chronic and asymptomatic conditions which can be detected by laboratory methods alone.

Unlike HIV infection, hepatitis B infection is a vaccine preventable disease, hence has the potential for eradication of the disease through wide coverage of immunisation.

Hepatitis B vaccine was introduced in the routine immunisation Programme of India in June 2002 and scaled-up nationwide in 2011 under Universal Immunisation Programme (UIP). Hepatitis birth dose was introduced in the national programme in 2008. Hepatitis B vaccine is provided as one of the pentavalent vaccines (against diphtheria, pertussis, tetanus, hepatitis B and Haemophilus influenzae B infections) administered at 6, 10 and 14 weeks, in addition to birth dose for institutional births. The birth dose is administered using a monovalent vaccine.

Several studies done before the introduction of Hepatitis B vaccine in the universal immunisation programme (2002) show a higher prevalence when compared to studies done after the introduction of vaccine (Table 2) [3,5,7-16]

In our study, the prevalence of HBV among Antenatal cases is 1.49%. This is comparable to the seroprevalence of 1.09% reported by Mishra S et al [3] and colleagues (in the year 2017), 1.01% reported in a study by Rajendiren S et al [16] (in 2017), 1.11% reported by Sibia P et al [15] (in 2016), 1.1% reported by Pandy et al [14] (in 2011).

As studies before and after the introduction of immunisation against Hepatitis B show a decline in the prevalence of the disease, our study also shows a prevalence of 1.49%, which is in concordance with other studies after 2002.

As regards to age, in present study high HBsAg carrier rate in pregnant women was found in the age group 26-30 years (1.92%), followed by the age group 21-25 years (1.45%), then the age group of 31-35 years (0.5%) as shown in Table 1.

A possible reason for the slightly higher HBsAg preva-lence in the 21-30 year-old age group is the fact that be-tween these ages, many females are likely to get married and become pregnant. They are therefore likely to attend antenatal care centers for the first time, and thus, can be easily picked up by screening.

Internationally, studies have reported various prevalence rates among pregnant women. In Brazil, a prevalence of 1.64%, France 0.29%, Italy 1.1%, Saudi Arabia 2.44%, Pakistan 4.6%, India 2.3% and Turkey 2.1%. In the Persian Gulf, rates of 7.1% and 1.5% in United Arab Emirate have been reported. In Africa, rates of 3% to 4% in Tunisia, 5.6% in Sudan and an astronomically high rate of 25% in Zimbabwe have been reported [17].

This study only screened for HBsAg in pregnancy in our area. As vertical transmission is responsible for HBsAg infections in child who are more likely to become carriers and later in life may present with cirrhosis and hepatocellular carcinoma,it may be enough if we screen all antenatal women and give combined immunization and immunoprophylaxis to the high risk infants born to seropositive mothers.[18] A combination of active and passive immune prophylaxis is the optimum strategy to prevent HBV infection in babies of HBsAg positive mothers. A combination of Hepatitis B immune globulin (HBIG) and Hepatitis B vaccination initiated within 24 hours of delivery have been shown to protect 85 to 95% of babies whose mothers were positive for HBsAg [19].

The study population included rural antenatal women, who were illiterate and had no awareness of transmission of the disease from mother to fetus. Social factors including early marriage and little formal education, lack of knowl-edge about hepatitis B infection and the importance of vaccination contribute significantly to a higher prevalence of STIs, including hepatitis B, among adolescent girls [20].

However in a country like India where a large number of deliveries are still non-institutional and laboratory facilities are not accessible to all, early detection of the disease is not feasible [21].The other contributing factor is, parents mistake the Hepatitis B infection for other causes of jaundice, metabolic diseases and neglect the infection. So there is a need for aggressive health education about the disease and vaccination through health workers, media and street plays to people.

Conclusion

The prevalence of hepatitis B infection among pregnant women attending Antenatal Clinic in our study is similar to the studies done after the introduction of vaccination in UIP. However even the prevalence of 1.49% can increase the pool of carriers and disease burden in the society. Hence aggressive health education and vaccination of all susceptible population are the main tools through which we can further reduce the prevalence of Hepatitis B virus infection in the society.

References

1. Kolawole OM, Wahab AA, Adekanle DA, Sibanda T, Okoh AI. Sero-prevalence of hepatitis B surface antigenemia and its effects on hematological parameters in pregnant women in Osogbo, Nige-ria. Virol J. 2012;9:317.
2. Umare A, Seyoum B, Gobena T, Haile Mariyam T. Hepatitis B Virus Infections and Associated Factors among Pregnant Women Attending Antenatal Care

Clinic at Deder Hospital, Eastern Ethiopia. PLoS ONE; 2016:1-11.

3. Mishra S, Purandare P, Thakur R, Agrawal S, Alwani M. Study on prevalance of hepatitis B in pregnant women and its effect on maternal and fetal outcome at tertiary care centre. Int J ReprodContracept ObstetGynecol 2017;6:2238-40.
4. Khakhkhar VM, Bhuva PJ, Bhuva SP, Patel CP, Cholera MS. Sero-prevalence of Hepatitis B amongst pregnant women attending the antenatal clinic of a tertiary care hospital, Jamnagar (Gujarat). Nat J Med Res 2012;2(3):362-365.
5. DwivediM, MisraSP, MisraV, Pandey A, Pant S, Singh R, Verma M. Seroprevalence of hepatitis B infection during pregnancy and risk of perinatal transmission. Indian J Gastroenterol 2011;30(2):66-71.
6. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr, Woods G, Procop G. Color Atlas and Textbook of Diagnostic Microbiology,6th edition, Philadelphia. Lippincott Williams and Wilkins Publishers; 2006.
7. Nayak NC, Panda SK, Zuckerman AJ, Bhan MK, Guha DK. Dynamics and Impact of perinatal transmission of Heoatitis B virus in North India. J Med Virol 1987;21(2):137-45.
8. Biswas SC, Gupta I, Ganguly NK, et al. Prevalence of hepatitis B surface antigen in pregnant mothers and its perinatal transmission. Trans Royal Soc Trop Med Hyg 1989;83:698-700.
9. Gupta I, Sehgal A, Seghal R, et al. Vertical transmission of hepatitis B in north India. J HygEpidemiol MicrobiolImmunol 1992;36:263-7.
10. Gill HH, Majumdar PD, Dhunjibhoy KR, et al. Prevalence of hepatitis B e antigen in pregnant women and patients with liver disease. J Assoc Physicians India 1995;43:247-8.
11. Mittal SK, Rao S, Rastogi A, et al. Hepatitis B: Potential of perinatal transmission in India. Trop Gastroenterol 1996;17:190-2.
12. Prakash C, Sharma RS, Bhatia R, et al. Prevalence in north India of hepatitis B carrier state amongst pregnant women. Southeast Asian J Trop Med Public Hlth 1998;29:80-4.
13. Chatterjee S, Ravishankar K, Chaterjee R, NarangA,Kinikar A. Hepatitis B prevalence during pregnancy. Indian Pediatr. 2009;46:1005-8.
14. Pande C, Sarin SK, Patra S. Prevalence, risk factors and virological profile of chronic hepatitis B virus infection in pregnant women in India. J Med Virol. 2011;83:962-7.
15. Sibia P, Mohi MK, Kumar A. Seroprevalence of Hepatitis B infection among Pregnant Women in One of the Institute of Northern India. Journal of clinical and diagnostic research: JCDR. 2016;10(8):QC08.
16. Sathiyakala R, Ushadevi G, Karthika J. Seroprevalence of Hepatitis B infection among pregnant women in South India. Int J ReprodContraceptObstetGynecol 2017;6:249-51.
17. Bernard UT. Hepatitis B surface antigenemia (HBsAg) among pregnant women in southern Nigeria. African Health Sci 2013;13(4):1139-1143.
18. Chakravarti D, Rawat M. A study on the perinatal transmission of the hepatitis B virus. Indian J Med Microbiol 2005;23(2):128-30.
19. MG Geeta and ARiyaz. Prevention of Mother to Child Transmission of Hepatitis B infection. Indian Pediatr 2013;50:189-192.
20. Bayo P, Ochola E, Oleo C, et al. High prevalence of hepatitis B virus infection among pregnant women attending antenatal care: a cross-sectional study in two hospitals in northern Uganda. BMJ Open 2014;4:e005889.
21. Ayatollahi J, Ayatollahi A, Ayatollahi R, MellatArdekani A, Shahcheraghi SH. Compliance with hepatitis B vaccination among adult males with sexually transmitted infections. Jundishapur J Microbiol. 2014;**7**(8):e11090.

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Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* Clinical Isolates at a Tertiary Care Hospital in Ambajogai

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Abstract

Introduction: Currently antibiotic resistance in bacterial populations is one of the greatest challenges to the effective management of infections. Infections caused by *Pseudomonas aeruginosa* are frequently life threatening & difficult to treat as it exhibits intrinsically high resistance to many antimicrobials & the development of increased, particularly multi-drug resistance in health care settings. *Objective:* The present study was done to determine the drug sensitivity pattern of *Pseudomonas aeruginosa* from various clinical specimens in our set up. *Materials and Method:* This study was conducted during October 2015 to May 2016. A total of 376 strains of Pseudomonas aeruginosa were isolated from various clinical specimens. *Pseudomonas aeruginosa* was identified by using standard microbiological techniques. Antimicrobial susceptibility patterns of all the isolates were carried out by Kirby- Bauer disk diffusion method as per CLSI guidelines. *Result:* A total 376 strains of *Pseudomonas aeruginosa* were isolated of which 267 (71%) were from indoor & 109 (29%) were from outdoor patients. Of the 376 isolates 206 were from males & 170 were from females. Majority of isolates of *Pseudomonas aeruginosa* were obtained from specimens of pus. Majority of the strains showed low level of susceptibility to Ceftazidime (38%), Ofloxacin (40.4%), Ciprofloxacin (42.1%), Piperacillin (47.7%) & Gentamicin (51%). We got good sensitivity with Tobramycin (82.1%) & with Amikacin (78.3%). All isolates were susceptible to Imipenem (100%). *Conclusion:* The result of our study suggests the occurrence of resistant strains of *Pseudomonas aeruginosa*. Periodic susceptibility testing should be carried out over a period of two to three years to detect the resistance trends & judicious, rational treatment regimen prescription should be followed by physician.

Keywords: Antibiotic Susceptibility Pattern; *Pseudomonas aeruginosa.*

Introduction

Pseudomonas aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that causes infections in hospitalized patient particularly in burn patients, ortho-paedicrelated infection, respiratory diseases, immunosuppressed and catheterized patients [1,2]. Nowadays more & more resistance of *Pseudomonas aeruginosa* are encountered in routine clinical practice, a serious problem, increase morbidity and mortality and also cost of treatment [3]. Members of thePseudomonad's genus are major agents of

nosocomial and community acquired infections, being widely distributed in the hospital environment where they are particularly difficult to eradicate [4]. It has also been observed that 28% of healthy people in hospital environment are carrier for*Pseudomonas aeruginosa* [5]. Mechanisms that cause antimicrobial drug resistance and multi-drug resistance in *Pseudomonas aeruginosa* are due to acquisition of resistance genes (e.g.those encoding beta-lactamase and amino-glycoside modifying enzymes) via horizontal gene transfer and mutation of chromosomal genes (target site, efflux mutations) are the target of the fluoroquinolones particularly ciprofloxacin [6]. *Pseudomonas aeruginosa* can survive harsh environmental conditions and displays intrinsic resistance to a wide variety of antimicrobial agents, two factors that facilitate the organism's ability to survive in hospital setting [7]. Biofilm formation in *Pseudomonas aeruginosa*, particularly in the case of pulmonary infections in patients with cystic fibrosis, contributes to its resistance to antimicrobial agents [8]. *Pseudomonas aeruginosa* demonstrates resistance to multiple antibiotics, thereby jeopardizing the selection of appropriate treatment [9]. Over period of time, we observed an increase in number of *Pseudomonas aeruginosa* among our laboratory isolates, and thereforethe present study was designed to find out the current antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* in our set up.

Materials and Method

Place

The study was carried out in the Department of Microbiology, Swami RamanandTeerth Rural Government Medical College and Hospital, Ambajogai, Beed.

Time Period

The study was of 8 months, conducted from October 2015 to May 2016.

Samples

Sampleswere collected from patients who are hospitalized for more than week with all aseptic precautions. The various clinical samples were included such as pus, sputum, urine, blood etc.

Isolation and Identification

The samples were further processed on the basis of their growth on routine MacConkey medium which showed lactose non-fermenting pale colonies which were oxidase test positive and on Nutrient agar pigmented and non-pigmented colonies with oxidase test positive. A battery of tests were performed that included Gram's staining, motility tests, sugar fermentation tests and biochemical tests such as urease and Phenyl pyruvic acid test and IMViC (indole, methyl red, Voges-Proskauer and citrate) tests for the identification of *Pseudomonas aeruginosa*.

Antibiotic Sensitivity Testing

Pseudomonas aeruginosa strains were subjected to antibiotic susceptibility testing on Mueller- Hinton agar by Kirby–Bauer disc diffusion method following National Committee for Clinical Laboratory standards (NCCLS) guidelines. The following antibiotics were tested: Imipenem(10 mcg / disc), Gentamycin(10 mcg / disc), Amikacin(30 mcg / disc), Ceftazidime(30 mcg / disc), Ciprofloxacin(5 mcg / disc), Ofloxacin(5 mcg / disc),Tobramycin(10 mcg / disc) and Piperacillin(100 mcg / disc). *Pseudomonas aeruginosa ATCC 27853* was used as control strain.

Result

A total of 376 strains of *Pseudomonas aeruginosa* were isolated and identified by standard microbiological procedures out of total 3387 samples. Out of total 376 strains 267 were from indoor and 109 from the outdoor patients, while 206 (55%) were from males and 170 (45%) from females.

Maximum isolates of *Pseudomonas aeruginosa*, 138 (36.7%) were from pus sample only, followed by sputum 98(26%) and urine 55 (13.8%) [Table 1].

Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* varied markedly. *Pseudomonas aeruginosa* isolates showed minimum susceptibility to Ceftazidime (38%) and maximum susceptibility to Tobramycin (82.1%). All isolates were sensitive to the carbapenem drug- Imipenem (100%).

Table 1: Sex wise distribution of the *Pseudomonas aeruginosa* isolates

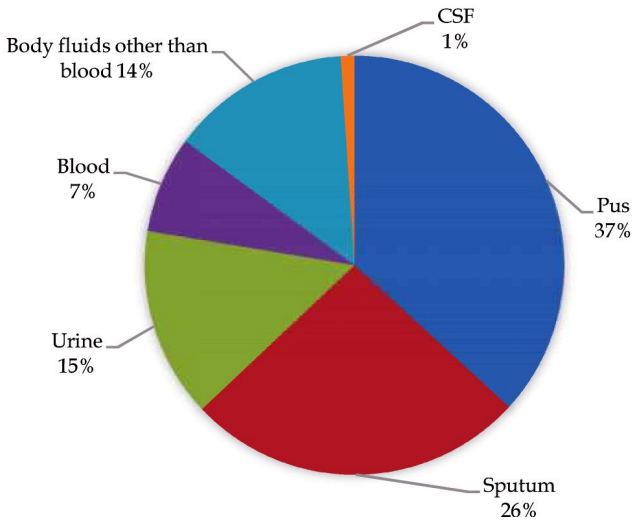
Sex	Total number	Percentage (%)
Male	206	55
Female	170	45
Total	376	100

Table 2: Isolation of *Pseudomonas aeruginosa* from various clinical specimens

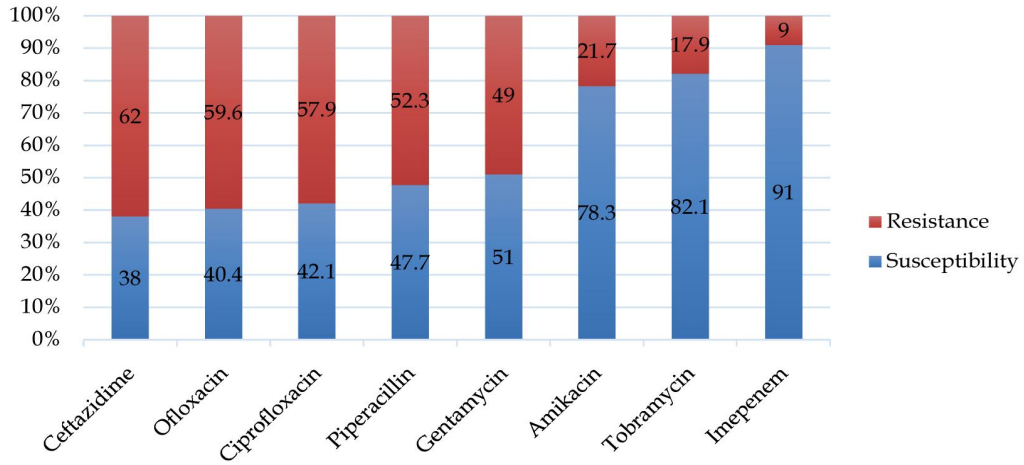
Specimen	Number of Isolates	Percentage (%)
Pus	138	36.70
Sputum	98	26.06
Urine	55	14.63
Blood	28	7.45
Body fluids other than blood	52	13.82
CSF	4	1.06
Total	376	100

Table 3: Antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from various clinical samples

Antibiotic	Sensitivity (%)
Ceftazidime	38
Ofloxacin	40.4
Ciprofloxacin	42.1
Piperacillin	47.7
Gentamycin	51
Amikacin	78.3
Tobramycin	82.1
Imipenem	100



Graph 1: Isolation of pseudomonas aeruginosa from various clinical specimens



Graph 2: Bar diagram showing the percentage of susceptibility and resistance of *Pseudomonas aeruginosa* to various antimicrobials

Discussion

Out of 3387 clinical samples of various natures, *Pseudomonas aeruginosa* was isolated from 376 samples i.e. the isolation rate was 11.1%, so it is undoubtedly an important nosocomial pathogen.Being an extremely adaptable organism, it can survive and multiply even with minimum nutrients, if moisture is available. The isolation rate of *Pseudomonas aeruginosa*in our study is comparable with study done by More S.R. et alwho mentioned isolation rate as 9.19% [10].

We can say that duration of stay is directly proportional to the rate of infection as out of 376 strains of *Pseudomonas aeruginosa* in our study, 267(71%) were from indoor and 109 (29%) were isolated from outdoor patients which is in correlation with Shampa Anupurba study who also mentioned the indoor patients to outdoor patients ratio that is 1:0.36 [11].

In the present study infections caused by *Pseudomonas aeruginosa* were more common in males (55%) compared to females (45%). This finding is in comparison with study done by Rajat Rakesh et al [4], Jamshid et al [12] and Rashid et al [13].

In our study, the maximum isolates of *Pseudomonas aeruginosa* were from pus / swab (36.7%), followed by sputum (26.06%). These results are in line with studies of Vijaya Choudhari et al [14] who mentioned that pus samples (35.3%) showed highest culture positivity followed by sputum (20.8%) and urine (13%).

In our study, majority of the isolates were susceptible to Tobramycin by 82% followed by Amikacin *Pseudomonas aeruginosa* isolates were found to be sensitive to Imipenem. This may be due to the 78.3% which is comparable with S. Shenoy [15] study. One striking feature in this study was that all the restricted use of Imipenem in this hospital. This is consistent with a report published in 2002 in Mangalore, India [15] but other studies have showed varying degrees of resistance to Imipenem in recent years [16,17,18,19].

In our study, the isolates were less susceptible to fluoroquinolones such as Ciprofloxacin (42.1%) similar findings had been reported in a study done in North Kerala [20].

This study shows that the clinical isolates of *Pseudomonas aeruginosa* are Multidrug resistance (resistance to ≥ 3 different classes of antibiotics tested) and are becoming resistant to commonly used antibiotics and gaining more and more resistance to newer antibiotics. The antimicrobial agents are losing their efficacy because of the spread of resistant organisms due to indiscriminate use of antibiotics, lack of awareness, patient noncompliance and unhygienic condition.

Conclusion

Results of the present study clearly demonstrated the occurrence of resistance to various antipseudomonal agents among the *Pseudomonas aeruginosa* isolates. Amikacin seems to be a promising therapy for Pseudomonal infection. Hence, its use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains. The problem of increasing resistance to *Pseudomonas aeruginosahas* limited the use of other classes of antibiotics like the fluoroquinolones, tetracycline's, macrolides and chloramphenicol [21].

Imipenem was the only anti-pseudomonal drug against which all isolates of *Pseudomonas aeruginosa*were fully sensitive. To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programsfor multidrug resistant organisms and infection control procedures need to be implemented. We suggest that there is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. The solution can be planned by continuous efforts of microbiologist, clinician, pharmacist and community to promote greater understanding of this problem.

References

1. Maria Renata, Gomes Franco, Helio Hell, Caiaffa Filho Marcelo, Nascimento Burattini, Flavia Rossi. Metallo- betalactamase among Imipemen resistant pseudomonas aeruginosa in a Brazilian university hospital. Clinics, 2010;65(9):825-829.
2. Tamil Selvi Sivannamaliappan, Murugan Sevanam. Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* from Diabetes Patients with Foot Ulcers. International Journal of Microbiology, 2011; Volume 2011; Article ID 605195.
3. Rajat Rakesh M. Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a tertiary care hospital, Ahmadabad. National Journal of Medical Research, 2012;2. ISSN:22494995.
4. H. Ravichandra Prakash, Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* Strains Isolated From Clinical Sources, JPBMS, 2012;14(05).

5. Kolmos HJ, Svendsen RN, Nielsen SV. The surgical team as resource of post - operative wound infections. J Hosp Infect 1997;35:207-14.
6. Chander Anil. Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* Clinical Isolates At A Tertiary Cars Hospital In Kathmandu ,Nepal.Asian Journal Of Pharmaceutical And Research, 2013;6 (Suppl 3).
7. Patricia M.Tille. Section 8, *Gram Negative Bacilli* and *Coccobacilli, Pseudomonas Burkholderia* and Similar organisms, Bailey And Scotts Diagnostic Microbiology, 13th edition, 2014.p.335-347.
8. Davies JC, Bilton D. Bugs, biofilms and resistance in cystic fibrosis. Respir care 2009;54:628-40.
9. Obritsch MD, Fish DN, Mc Laren R, Jung R. National Surveillance of Antimicrobial Resistance in *Pseudomonas aeruginosa* isolates obtained from Intensive Care Unit Patients from 1993 to 2002. Antimicrob Agents Chem. 2004;48:4606–10.
10. More S.R., Raut S.S ,Gujar V.M, Rathod V.S, Rajhans V.V, Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated From Various Clinical Samples At A Tertiary Care Centre. IJSHR, 2015 Jan;5.
11. Shampa Anupurba. Antimicrobial Suseptibility Of *Pseudomonas aruginosa* Isolated From Wound Infections. Indian J Dermatol 2006:51(4):286-8.
12. Jamshaid A K, Zafar I, Saeed U R, K. Farzana, Abbas K.Prevalence and resistance patterns of *Pseudomonas aeruginosa* against various antibiotics. Pak. J. Pharm. Sci. 2008 Jul;21(3):311-315.
13. Rashid A, chowdhury A, Sufi HZ R, Shahin A B, Naima M. Infections by *Pseudomonas* and antibiotic resistance pattern of the isolates from Dhaka Medical college Hospital. Bangladesh J Med Microbiol 2007;01(02):48-5.
14. Chaudhary V et al. Antibiotic resistance patterns of Pseudomonas aeruginosa in a tertiary care hospital in central India. International journal of Medical science and public health. 2013;2(02):386-389.
15. Shenoy S, baliga S, Saldanha DR, Prashanth HV. Antibiotic sensitivity patterns of *Pseudomonas aeruginosa* strains isolated from various clinical specimens. Indian J Med Sci. 2002 Sep;56(9)427-30.
16. Mohanasoundaram KM. The antibiotic resistance pattern in the clinical isolates of *Pseudomonas aeruginosa* in a tertairy care hospital; 2008-2010 (A 3 year study). J ClinDiagn Res 2011;5(3):491-94.
17. Arora D, Jindal N, Kumar R, Romit. Emerging antibiotic resistance in *Pseudomonas aeruginosa*. Int J Pharm PharmSci 2011;3(2):82-4.
18. Javiya JA, Ghatak SB, Patel KR, Patel JA. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Gujarat, India. Indian J Pharmacol 2008;40(5):230-34.
19. Al-Kabsi AM, Yusof MYBM, Sekaran SD. Antimicrobial resistance pattern of clinical isolates of *Pseudomonas aeruginosa* in the University of Malaya Medical Center, Malaysia. Afr J Microbiol Res 2011;5(29):5266-72.
20. Ahmed SM, Jakribettu RP, Kottakutty S, Arya B, Shakir VPA. An emerging multi-drug resistant pathogen in a tertiary care centre in North Kerala. Annals Biol Res 2012;3(6):2794-99.
21. Chambers HF. General Principles of antimicrobial therapy. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. Mc-Graw Hill: Medical Publishing Division; 2006.p.1095–110.

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Study on Bacterial Profile of Urinary Tract Infection and Antimicrobial Susceptibility Pattern among Pregnant Women: A Study from a Tertiary Care Hospital

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Abstract

Introduction: Urinary tract infections (UTI) are the most common infections in clinical practice. They can have serious complications on maternal and fetal outcome when they occur in pregnancy. Screening all pregnant women for significant bacteriuria can prevent the complications of UTI in pregnancy. *Aims and Objectives:* To studythe bacterial profile of urinary tract infections and the antimicrobial susceptibility patterns among pregnant women. *Material and Methods:* This was a prospective hospital basedstudy carried out in the Department of Microbiology, Deccan College of Medical Sciences, Hyderabad, Telangana, over a one year period from April 2016 to March 2017. *Results:* A total of 235 urine samples were received from pregnant women and processed in Microbiology laboratory. 110 urine samples showed significant bacteriuria. The most commonly isolated bacteria were E. coli 80 (72.7%), K. pneumoniae 7 (6.3%), S. aureus 13 (11.8%), P. aeruginosa 5(4.5%), Coagulase negative staphylococci 5(4.5 %). *Conclusion:* This study showed that prevalence of UTI in pregnant women was 36.2%. It was also observed that E. coli (72.7%) was the most frequently isolated bacteria. All pregnant women should be screened for UTI with a urine culture and treated with antibiotics if the culture is positive.

Keywords: Urinary Tract Infection; Pregnancy; Urine Culture Antimicrobial Susceptibility.

Introduction

Urinary tract infections are the most common infections in clinical practice [1]. Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is a frequent cause of hospital visits [2]. It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually [3]. The most common pathogenic organisms of UTI are Escherichia coli, Staphylococcus saprophyticus and less common organisms are Proteus sp., Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococci and Candida albicans [4]. There are some structural and functional urinary tract changes, typical for pregnancy. In about 80% of pregnant women dilation of the urinary tract combined with slight hydronephrosis is observed, caused partly by a reduction in smooth muscle tone with slowing of ureteral peristalsis, and partly by urethral sphincter relaxation. This may be due to high levels of circulating progesterone [5]. Urinary tract infections (UTIs) in pregnant women pose a clinical problem and a great challenge for physicians.

Although the incidence of bacteriuria in this population is only slightly higher than in non-pregnant women, its consequences for both the mother and the fetus are severe. There is a much higher risk (up to 40%) of progression to pyelonephritis, and possibly increased risk of pre-eclampsia, premature birth and low neonatal birth weight. The increased level of progesterone in pregnancy is thought to contribute to the increased risk of UTI [5-11].

Aim of the Study

The aim of the study was to assess bacterial profile of urinary tract infections and their antimicrobial susceptibility patterns among pregnant women attending antenatal clinic at Deccan College of Medical Sciences and Owaisi Research Centre, Hyderabad.

Materials and Methods

This was a prospective hospital based study done from April 2016 to March 2017 (one year). A total of 235 urine samples were studied from pregnant women between the ageof 18 to 40 years who were attending antenatal clinic at Deccan College of Medical Sciences and Owaisi Research Centre. The clinical details including name, age, and address of the patients wererecorded.

Early morning clean-catch midstream urine sample was collected from each pregnant woman into a wide-mouthed sterile screw capped container. Samples were labeled and analyzed within 30 minutes of collection.

The collected urine samples were inoculated on CLED (cysteine lactose electrolyte deficient agar) agar plates and the culture media were incubated aerobically at 37°C.

The urine culture plates were examined for pure growth determined by morphologically same type of colonies and colony counts for determination of significant and insignificant growth.

Urine culture was then performed. Identification and characterization of isolated bacteria included Gram stain followed by microscopic examination, motility test and biochemical tests as described by other workers [12-15].

For drug susceptibility test, Mueller Hinton agar (MHA) was used and commercial antibiotic multidisc were used as described by Al-ghalibiet al [16]. Antibiotic discs, Amoxicillin (AMX) 25μg, Augmentin® (AUG) 10μg, Cotrimoxazole (COT), Ciprofloxacin (CIP) 10μg, Gen-tamycin (GEN)10μg, Imipenim (IPM)10μg, Levofloxacin (LEV) 30μg, Meropenem (MEM) 10μg, Nalidixic acid (NA 30ug), Oflox-acin (OFL) 5μg, were used. Zone of inhibition was measured to determine the level of susceptibility of isolates to the antibiotics. Data obtained in this study were analyzed using SPSS version16.0.

A growth of $\geq 10^5$ colony forming units/ml was considered as significant bacteriuria.

Results

About 235 urine samples were collected from all pregnant women during the study

Among 235 cases, 110/235 (46.8%) cases were positive for bacteria and 125/235 (53.1%) were negative.

On microscopic examination pus cells were predominant finding in 85 (77.2%) cases.

Table 1: Distribution of UTI in different age groups

Age (years)	No. of Cases	No. of Positive Cases	No. of Negative Cases
18-22	37	16	21
23-27	61	21	40
28-32	89	59	30
33-37	42	11	31
38-40	06	03	03
Total	235	110	125

Table 2: Microscopic examination of urine samples

Isolates	No. of Positive Samples	(%)
Pus cells	85	77.2%
Red blood cells	10	9.09%
Yeast cells	15	13.6%
Total	110	100 %

Table 3: Bacteria isolated from the urine samples

Isolated Organisms	Number of Positive Sample	Percentage (%)
E. coli	80	72.7%
S.aureus	13	11.8%
K.pnuemoniae	07	6.3%
Coagulase negative staphylococci	05	4.5%
Acinetobacter	02	1.8%
Enterococci	03	2.7%
Total	110	100 %

Table 4: Antibiotic sensitivity pattern of Gram Negative bacteria

Gram Negative bacteria	AMC	AMP	CIP	CTX	CTR	GEN	NIT	AK	NX	IPM	T
E.coli (80)	35(43.7%)	10(12.5%)	47(58.7%)	42(52.5%)	36(45%)	447(58.7%)	74(92.3%)	45(56.2%)	47(58.7%)	80(100%)	29(36.8%)
K. Pnuemoniae (7)	0	1(14.2 %)	1(14.2 %)	3(42.8%)	3(42.8%)	2(28.5%)	3(42.8%)	6(85.7%)	5(71.4%)	7(100%)	5(71.4%)
Acinetobacter (2)	0	0	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	2(100%)	1(50%)

Table 5: Antibiotic sensitivity pattern of Gram Positive bacteria

Gram positive bacteria	AMC	AMP	CIP	CTX	CTR	GEN	NX	C	COT	LZ	P	E	T
S.aureus (13)	7(53.8%)	3(23%)	9 (69.2%)	7 (53.8%)	8 (61.5%)	5 (38.4%)	9 (69.2%)	7 (53.8%)	10 (76.9%)	13 (100%)	3 (23%)	5 (38.4%)	7 (53.8%)
CONS(5)	2(40%)	1(2%)	2(40)	2 (40%)	3 (60)	2 (40%)	2 (40%)	4 (80%)	3 (60%)	5 (100%)	1 (20%)	1 (20%)	3(60%)
Enterococci (3)	0	0	1 (33.%)	0	0	1 (33.3%)	0	0	1 (33.3%)	3 (100%)	0	0	0

Table 6: Incidence of UTI by trimester period

Trimester Period	No. of Cases	Percentage (%)
I trimester (1st 3 months)	10	9.0 %
II trimester (2nd 3 months)	40	36.3%
III trimester (3rd 3 months)	60	54.5%
Total	110	100 %

The most commonly isolated bacteria were E. coli 80 (72.7%) followed by S. aureus 13 (11.8%).

Gram-negative bacteria were more prevalent (80.9%) than Gram-positive bacteria (19%).

AMP-Ampicillin, AMC-Amoxiclav, CTX-Cefotaxime, CTR-Ceftriaxone, CIP-Ciprofloxacin, GEN-Gentamicin, AK-Amikacin, NIT-Nitrofurantoin, T-Tetracycline, NX-Norfloxacin, IPM-Imipenem.

CONS- Coagulase negative Staph, AMP-Ampicillin, AMC-Amoxiclav, CTX-Cefotaxime, CTR-Ceftriaxone, CIP-Ciprofloxacin, GEN-Gentamicin, AK-Amikacin, NIT-Nitrofurantoin, T-Tetracycline, NX-Norfloxacin, IPM-Imipenem.

All Gram negative bacteria 80 (100%) in our study were sensitive to Imipenem which is not a safe drug in pregnancy.

E. coli 74 (92.3%) and Klebsiella 6(85.7%) were sensitive to nitrofurantoin which can be used in pregnancy.

Only 10 (12.55%) E.coli were sensitive to Ampicillin

In the present study, women in their 2nd and 3rd trimester were found to have the higher incidence of UTI; 36.3% and 54.5%, respectively.

Discussion

In the present studya total of 235 urine samples from pregnant women were studied for bacteriuria. The rate of positive samples was 46.8%. In a study by Samaga et al [17] (n=124), 36.2% samples yielded significant bacterial growth. Jain et al [18] 150 urine specimens from pregnant women were studied for bacteriuria. In the study by Manjula et al [19] (n=417), 49.4% showed significant bacteriuria.

In the present study, on microscopic examination pus cells were predominant 85 (77.2%) followed by yeast cells 10(9.09%) and red cells 15 (13.6%). In the study by Manjula et al [19] urine microscopy revealed >10 pus cells / high power (40×) field. In a study by Battikhi et al [20] (n=123)microscopic observation of midstream urine samples revealed white blood cells (pus cells) in 26% cases and red blood cells in 9.7% cases.in our study, the age groups of 28-32 (53.6%) years and 23–27(19%) years showed highest incidence of bacteriuira. In the study by Manjula et al [19] women in the age groups of 21-25 and 36-40 years showed highest incidence.

In the present study, the most commonly isolated bacteria were E. coli 80 (72.7%) followed by S. aureus 13 (11.8%), K. pneumoniae 7 (6.3%), S. aureus 13 (11.8%), P. aeruginosa 5(4.5%) and Coagulase negative staphylococci 5 (4.5%). Gram-negative bacteria were more prevalent (80.9%) than Gram-positive bacteria (19%). Jain et al [18] observed significant bacteriuria, >105 colony forming units (CFU) per ml in 52 (34.6%) patients among 150 patients. Various bacteria isolated from the urine sample in their study were E. coli (44.2%), K. pneumoniae (19.2%), S. aureus (21.1%), P. aeruginosa (7.6 %) and E. aerogens(7.6 %). In the study by Manjula et al [19] majority of the isolates (99%) were Gram negative bacteria which included Escherichia coli (56.7%), Klebsiellasps (19.9%), Pseudomonas sps(6.3%), Proteus sps (5.8%), Enterobacter sps (3.8%), Citrobactersps (1.4%), Enterococcus sps (0.9%), and other NFGNB (4.8%). Battikhi et al [20] observedprevalence of gram negative and gram positive bacteria to be 37 (69.8%) and 14 (26.4%) respectively. E. coli showed the highest prevalence rate followed by S. Aureus 11(30.8%) and K. pneumoniae 7(13.2%).

E. coli and S. aureus were the most common isolates in all age groups [3,8] however; P. aeruginosa was isolated from age group 38-42 years exclusively.In the study by Samaga et al [17] E.coli (42.2 %) was isolated as predominant pathogen followed by Staphylococcus aureus (17.8%), CONS (15.5%), Klebsiella (11.1%), Enterococci (6.7%) and Acinetobacter (6.7%).

In the present study, women in their 2nd and 3rd trimester were found to have a higher incidence of UTI; 36.3% and 54.5%, respectively. Manjula et al [19] have reported the prevalence of UTI by gestational age (age of pregnancy) as lowest in the 3rd month 25% followed by 29% in 5th month and highest, 70.2% in 7th month of pregnancy. While the incidence of UTI if taken by trimester was 43.3% and 54.1% in 2nd and 3rd trimester respectively than in the first trimester (25%) [18] also observed that women in their 2nd and 3rd trimester were found to have a higher incidence of UTI; 32.6% and 48%, respectively. Battikhi et al [20] also observed thatwomen in their 2nd and 3rd trimester were found to have a higher incidence of UTI; 32.6% and 48%, respectively.

Jain et al [18] studied the antibiotic sensitivity pattern of isolated organisms. They found E. coli was highly sensitive to nitrofurantoin, K. pneumoniae to ciprofloxacin, E. aerogensto ceftazidine ,P. aeruginosa to ceftazidime and S. aureus was highly sensitive to levofloxacin and nitrofurantoin.

Battikhiet al [20] studiedantibiotic sensitivity test and observed 100.0% activity for meropenem, imipenem and levofloxacin. They were the most ac-tive antibiotics and the rate of multidrug resistance was (>50%).

Conclusion

UTI is one of the most common bacterial infections during pregnancy. Most of the UTIs during pregnancy can lead to complications and poor fetal outcome if untreated.

The most commonly isolated bacteria were E. coli and S. aureus and Gram-negative bacteria were more prevalent than Gram-positive bacteria. Early diagnosis and treatment of UTI in antenatal women should be done to prevent complications and improve pregnancy outcomes for both the mother and the baby.

References

1. Noor N, Ajaz M, Rasool SA, Pirzada ZA. Urinary tract infections associated with multidrug resistant enteric bacilli, characterization and genetical studies. Pak J Pharm Sci. 2004;17:115-123.
2. Ronald AR, Pattulo MS. The natural history of urinary infection in adults. Medical clinics of North America 1991;75:299-312.
3. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis. 2004;38:1150–1158.

4. Salek, SB. Infective syndrome in medical microbiology. 1992 4th edition, pp. 740.
5. Schnarr J, Smaill F. Asymptomatic bacteriuria and symptomaticurinary tract infection in pregnancy. Eur J Clin Invest 2008;38(Suppl. 2):50-7.
6. Farkash E, Wientraub AY, Sergienko R, Wiznitzer A, Zlotnik A, Sheiner E. Acute antepartumpyelonephritis in pregnancy: a critical analysis of risk factors and outcomes. Eur J ObstetGynecolReprodBiol 2012; 162:24-7.
7. Gravett MG, Martin ET, Bernson JD, Bernson JD, Khan S, Boyle DS, et al. Serious and life-threatening pregnancy-related infections: opportunities to reduce the global burden. PLoS Med 2012;9(10):1-7.e1001324.
8. Foxman B. Epidemiology of urinary tract infections: incidence,morbidity, and economic costs. Am J Med 2002;113:5-13.
9. Mazor-Dray E, Levy A, Schlaeffer F, Sheiner E. Maternal urinary tract infection: is it independently associated with adverse pregnancy outcome? J Matern Fetal Neonatal Med 2009;22:124-32.
10. Bolton M, Horvath DJ, Li B, Cortado H, Newsom D, White P, et al. Intrauterine growth restriction is a direct consequence of localized maternal uropathogenic Escherichia coli cystitis. PLoS ONE 2012;7(3):1-9.e33897.
11. Jeyabalan A, Lain KY. Anatomic and functional changes of the upper urinary tract during pregnancy.UrolClin North Am 2007;34:1-6.
12. Osuala FOU, Anyadoh SO, Nnoli MC. Antibacterial activity of two local medicinal plants, Utazi (Gongronenalatifolium) and Nchuanwu (Ocimiumgratissimium). International Journal of Natural and Applied Sciences 2005;1(1):36 -39.
13. Todar K (2009) Todar's Online Textbook of Bacteriology. (2009) Wisconsin, USA.
14. Smith PJ, Morris AJ, Reller LB. Predicting urine culture results by dipstick testing and phase contrast microscopy. Pathology 2003;35(2):161-165.
15. Cheesbrough M. Medical laboratories manual for tropical countries. 2006;(2nd edn), Cambridge University Press, London, UK, 2006.p.479.
16. Al-ghalibi SM, Al-Moayad E, Al-Jaufy A. Bacterial urinary tract infection among pregnant women in Sana'a city-Yemen. Arab Gulf J of Scientific research 2007;25(1-2): 23.
17. Samaga MP. Bacteriological Profile of Urinary Tract Infections in Pregnant Women.Indian J Microbiol Res 2016;3(1):17-21.
18. Jain M, Farooq U, Begum R, Tak V, Verma A, Gupta J. Bacteriological Study of Urinary Tract Infection in Ante-natal Patients in Teerthanker Mahaveer University. International Journal of Scientific Study 2015;3(3).
19. Manjula NG, Math GC, Patil SA, Gaddad SM, Shivannavar CT.Incidence of Urinary Tract Infections and Its Aetiological Agents among Pregnant Women in Karnataka Region. Advances in Microbiology 2013;3:473-478.
20. Battikhi MN, Battikhi QG.Correlation of Urinary Tract Infection Pathogens, Antibiogram and Age Group in Pregnant Women,Journal of Microbiology and Experimentation 2015;2(4).

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Speciation and Resistotyping of Coagulase Negative Staphylococci in Clinical Isolates in Kerala Medical College, Palakkad

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Abstract

Introduction: Coagulase negative staphylococci are important causative agents of 10% of pyogenic infections in hospitals, which include a range of infections like surgical wound sepsis, bacteremia, native valve endocarditis (NVE) and prosthetic valve endocarditis, osteomylitis, pyoarthritis, peritonitis, mediastinitis, prostatitis, infection of vascular grafts and pacemakers. *Materials and Methods:* 105 samples are collected from infected post operative cases, infected burns, infected wounds from traumotology unit, diabetic foot, gangrene, nonhealing ulcers, pyoderma, and impetigo, patients with intravenous cannulae. *Results:* of 105 samples tested 50 (47.61%) of CNS are isolated, Out of 50 isolates of Coagulase negative staphylococci 24 (48%) isolates were identified as S.epidermidis. Out of 34 community isolates, 13 (38.23%) were sensitive to Penicillin and. 11(61.76%)were resistant 13 (75.47%) were sensitive to Oxacillin and 3(13.23%) were resistant. *Conclusion:* In the present study CNS was the most predominant organism (105/50; 47.61%). Most of CNS isolates were from the hospital infections (68%). Coagulase negative Staphylococci have been increasingly gaining importance in hospital infections, compared to E. coli, Klebsiella, MRSA and Pseudomonas. Majority of the isolates were from IV catheters (76.19%), stressing the need for more aseptic precautions in ICU settings.

Keywords: I.V.; MRSA; CNS.

Introduction

The importance of coagulase negative Staphylococci (CNS) in causing human infections is well documented (Pulverer and Pillich, 1971) [1]. Coagulase negative staphylococci are important causative agents of 10% of pyogenic infections in hospitals, which include a range of infections like surgical wound sepsis, bacteremia, native valve endocarditis (NVE) and prosthetic valve endocarditis, osteomylitis, pyoarthritis, peritonitis, mediastinitis, prostatitis, infection of vascular grafts and pacemakers, infective intravascular catheters, cerebrospinal fluid shunts, orthopedic devices and urinary tract infections.

Normally commensals on the skin and mucous membrane, coagulase negative staphylococci become opportunistic pathogens in conditions of lowered resistance in the local area. Establishment of causal relationship is important in treatment. Repeated isolation of the same organism from the lesion establishes the etiological relationship.

Coagulase test is the arbitrary test to divide the staphylococci into two groups. Group one consists of coagulase positive staphylococci, which are the established pathogens. Group two consists of

coagulase negative staphylococci, which are commensals and opportunistic pathogens.

Till a few decades ago, infections caused by these organisms were restricted to skin / wound infections and UTI in sexually active females. Coincident with remarkable developments in disease diagnosis and treatment, as well as the increased incidence of device associated infections caused by coagulase negative staphylococci, the importance of these organisms in human infection has remarkably increased in the recent times. As this organism occupies more than 10% of hospital infections, antibiotic sensitivity pattern also showed a change and increased degree of antibiotic resistance is being documented. There are a number of species of coagulase negative staphylococci, but only a few of them are predominant in causing opportunistic infections.

Coagulase negative staphylococci isolated from various specimens received from these hospitals are speciated and their antibiogram is studied. Since most of the infections caused by coagulase negative staphylococci are hospital associated, it is extremely relevant to study the resistance pattern so that it will serve as a useful guide to the health care providers. In a recent study (Banerjee et al. 1991) [2] resistance to betalactam antibiotics has been reported in coagulase negative staphylococci as well. Hence the study is attempted to speciate the clinical isolates of coagulase negative staphylococci and study their antibiogram with special reference to methicillin and vancomycin resistance.

Material and Methods

Sample Collection

The study was conducted in Kerala medical college & Hospital, Pallkad,Kerala The samples were collected from outpatients and inpatients of surgical, medical, orthopedic wards, 105 samples are collected from infected post operative cases, infected burns, infected wounds from traumotology unit, diabetic foot, gangrene, nonhealing ulcers, pyoderma, and impetigo, patients with intravenous cannulae were chosen as subjects of study after obtaining approval from the institutional Ethics committee and written informed consent was taken from the patients. The co morbid, immunosupressed patients, Patients with Diabetes mellitus patients with Malnutrition and on Steroid therapy, patients with Hypoprotinemia, patients with Malignancies and on Anti-malignancy drugs were completely excluded. Serous, serosanguinous or purulent discharge from the ulcers or wounds is collected with sterile swabs from the base of the lesions, without touching the surrounding area of skin. In case of spreading lesions of skin and subcutaneous tissue (such as progressive gangrene) the material is collected from the active margins of the lesions, rather than from central portion. When the exudate was minimal, gentle pressure was applied at the base of the lesion and the expressed discharge was collected with the swab. Care was taken to avoid topical application of any antibiotics, at least 24hrs before collection of the sample. Two swabs were collected from each patient, one for making smears and another for culture. Swabs sterilized by autoclaving was used to collect specimens in preference to swabs sterilized by hot air oven .

The samples were processed as per the standard reference procedures (Koneman 2006) [3] Gram positive cluster forming staphylococci which are catalase positive oxidase negative bacitracin resistant furozolidone sensitive and fermentative by the OF test are identified as staphylococcal the staphylococci strains were subjected to slide and tube coagulase test and those strains, which are negative by both methods are identified as coagulase negative staphylococci.

As no single table in the standard references was completely comprehensive to identify most of the common species, a table was prepared using the standard reference texts and journals. This table helped in the identification of all the species of CNS isolated .

The following biochemical tests were used.

1. Ornithine decarboxylase
2. Urease
3. Novobiocin susceptibility
4. Polymyxin B
5. Trehalose fermentation
6. Mannitol fermentation
7. Lactose fermentation
8. Voges proskauer

Antibiogram of isolates was performed using Kirby Bayer method (Clinical and laboratory standards institute CLSI guidelines). The antibiotic discs used were

Amoxicillin 30ug

Penicillin 10 units

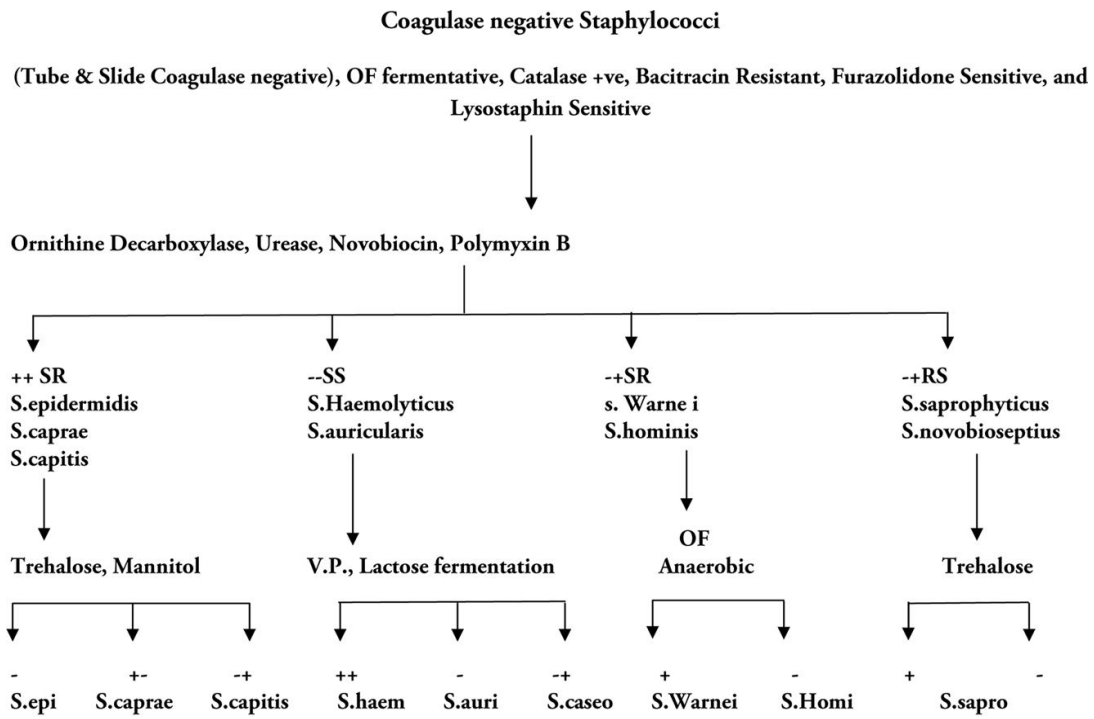
Oxacillin 1 mcg

Ciprofloxacin 5 mcg

Cotrimoxazole 25 mcg

Vancomycin 30 mcg

Results are tabulated



(Honeman 2006. Forbes 2007 R.Goyal 2006)

Results

Table 1 showing number and percentage of CNS isolates in the present study out of 105 samples tested 50 (47.61%) of CNS are isolated

Table 2 shows Speciation of Coagulase negative staphylococci isolates. Out of 50 isolates of Coagulase negative staphylococci 24 (48%) isolates were identified as S.epidermidis. 10 (20%) were identified as S.saprophyticus. 8(15%) were identified as S.haemolyticus. 7 (14%) were identified as S.hominis. 1 (3%) were identified as S caprae.

Table 3 showing distribution of hospital & community isolates of Coagulase negative staphylococci. Out of 50 samples, hospital isolates are 34 (68%), community isolates of Coagulase negative staphylococci are 16 (32%).

Table 4 shows isolates of Coagulase negative staphylococci from various sources. Out of 105 samples processed, 50 Coagulase negative staphylococci have been isolated, of which 21 were from cannulae and 16 (76.19%) Coagulase negative staphylococci were isolated. Out of 34 wound samples processed 19 (55.88%) were Coagulase negative staphylococci. Out of 27 urine samples processed 7 (27%) were Coagulase negative staphylococci. Out of 19 blood samples processed 7 (35.89%) were Coagulase negative staphylococci . Out of 3 samples of CSF only zero Coagulase negative staphylococcus isolated.

Table 1: Number and percentage of Coagulase negative staphylococci isolates in the present study

Total number of test Samples	Coagulase Negative Staphylococci	
	Number	Percentage
105	50	47.61

Table 2: Speciation of Coagulase negative staphylococci isolates

S. No.	Name of the Species	Number	Percentage
1.	S. epidermidis	24	48
2.	S. saprophyticus	10	20
3.	S. haemolyticus	8	15
4.	S. hominis	7	14
5.	S. caprae	1	3

Table 5 shows Sex wise distribution of Coagulase negative staphylococci isolates. 26(51%) of isolates were from males and 24 (49%) were from females.

Table 6 shows Age wise distribution of Coagulase negative staphylococci isolates. 4 (8%) of Coagulase negative staphylococci were seen in age group of 0-15 years. 8 (15%) of Coagulase negative staphylococci were seen in age group of 16-40 years. 39 (77%) of Coagulase negative staphylococci were seen in age group of more than 40 years.

Table 7 showing Antibiogram of hospital isolates of Coagulase negative staphylococci under study.

Out of 34 community isolates, 13 (38.23%) were sensitive to Penicillin and 11 (61.76%) were resistant.

Table 3: Distribution of hospital and community isolates of Coagulase negative staphylococci

S. No	Nature of isolates	Number	Percentage
1	Hospital	34	68
2	Community	16	32

Table 4: Isolates of Coagulase negative staphylococci from various sources

S. No	Sample	Number of samples processed	Number of CNS isolates	Percentage
1	IV catheter	21	16	76.19
2	Wound	34	19	55.88
3	Urine	27	7	27
4	Blood	19	7	35
5	CSF	3	0	0

Table 5: Sex wise distribution of Coagulase negative staphylococci isolates

S. No	Sex	Number of isolates	Percentage
1.	Male	26	51
2.	Female	24	49

Table 6: Age wise distribution of Coagulase negative staphylococci isolates

S. No	Age group	Number of CNS isolates	Percentage
1	0-15	4	8
2	16-40	8	15
3	> 40	39	77

Table 7: Antibiogram of hospital isolates of Coagulase negative staphylococci under study

S. No	Antibiotic	Resistant		Sensitive	
		Number	Percentage	Number	Percentage
1	Penicillin	11	61.76	7	38.23
2	Oxacillin	3	13.23	13	75.47
3	Ciprofloxacin	12	70.58	05	29.41
4	Vancomycin	4	23.52	13	76.47
5	Co trimoxazole	15	86.76	2	13.23
6	Amoxycillin	14	82.35	3	17.64

Table 8: Antibiogram of community isolates of Coagulase negative staphylococci under study

S. No.	Antibiotic	Resistant		Sensitive	
		Number	Percentage	Number	Percentage
1	Penicillin	5	34.37	10	65.62
2	Oxacillin	1	9.37	15	90.62
3	Ciprofloxacin	7	30.62	9	59.37
4	Vancomycin	8	46.87	5	53.13
5	Co trimoxazole	6	18.75	13	81.25
6	Amoxycillin	14	43.75	18	56.25

13 (75.47%) were sensitive to Oxacillin and 3 (13.23%) were resistant. (29.41%) were sensitive to Ciprofloxacin and 12 (70.58%) were resistant. 13 (76.47%) were sensitive to Vancomycin and 4 (23.52%) were resistant. 2 (13.23%) were sensitive to Cotrimoxazole and 15 (86.76%) were resistant. 3 (17.64%) were sensitive to Amoxycillin and 14 (82.35%) were resistant

Table 8 showing Antibiogram of community isolates of Coagulase negative staphylococci under study.

Out of 32 community isolates, 21(65.62%) were sensitive to Penicillin and 11 (34.37%) were resistant.

29 (90.62%) were sensitive to Oxacillin and 3 (9.37%) were resistant. 19 (59.37%) were sensitive to Ciprofloxacin and 13 (30.62%) were resistant. 17 (53.13%) were sensitive to Vancomycin and 5 (46.87%) were resistant. 26 (81.25%) were sensitive to Cotrimoxazole and 6 (18.75) were resistant. 18 (56.25%) were sensitive to Amoxycillin and 14 (43.75%) were resistant.

Table 9 shows Frequency of clinically significant Coagulase negative staphylococci under study.

Among total of 50 isolates of Coagulase negative staphylococci 24 identified as S. epidermidis, among which 9 are from IV cannulae, 6 are from blood, 4 each from urine & wounds and only one from CSF. Among the 10 isolates of S. saprophyticus, 5 are from IV cannulae, 4 from wound and one from urine. Among 7 isolates of S. haemolyticus, 6 are from wound, 1 from urine and zero from IV cannula.

Among 7 isolates of S. hominis, 5 are from wound, 1 each from blood and urine and one from IV cannula. Among 1 isolates of S. caprae, 1 are from wound and one from urine.

Table 9: Frequency of clinically significant Coagulase negative staphylococci under study

S. No	Name of the species	Total number	Wound	Catheter	Blood	CSF	Urine
1.	S.epidermidis	24	4	9	6	0	4
2.	S.saprophyticus	10	4	5	-	-	0
3.	S.haemolyticus	7	6	0	-	-	1
4.	S.hominis	7	5	0	1	-	1
5.	S caprae	1	1	-	-	-	-

Discussion

The clinical significance of CNS continues to increase as recent advances in medical practice lead to more invasive procedures, using introduction of synthetic material in to the body. The most vulnerable group to infection by CNS are hospitalized patients, especially those who are premature, very young or old and those who are immunocompromised and / or suffering from chronic debilitating illness or malignancy. Thanks to the progress in medical research, we are having more number of immunocompromised people, who can be managed for longer periods. All these groups are highly vulnerable to infections by CNS. Probably the only exception is young sexually active females who suffer from urinary tract infection due to S saprophyticus.

Since CNS is a commensal found on human body, establishment of causal; relationship to clinical disease is a serious challenge to the microbiology laboratory. If only adequate care is taken for accurate specimen collection and care taken to ensure the quality of the specimen, the challenge can be minimized. If the same isolate is obtained repeatedly from the same sample collected again and again, the etiological relationship becomes stronger. It can be further strengthened by genotyping of the isolates. However genotyping is not accessible to most of the microbiology laboratories. In the present study (Table 1) 50 CNS isolates were obtained by processing 105 clinical samples (47.61%) 68% were hospital isolates (Table 3), these results indicate the prominent place occupied by CNS in infections and more so in the hospital infections. The most predominant organisms causing hospital infection in the present times are E.Coli (Raksha et al 2003) [4], 102 Klebsiella (Casewell & Philips 1981) [5] 13, Pseudomonas and MRSA .The gradual increase of the importance of CNS in the recent times envisages the future place of CNS among microorganisms causing hospital infections. It need not be a surprise if it occupies the first place in the next decade to come.

The percentage isolation of CNS from clinical samples is not available in similar studies Show et al 2005, 112 have collected 205 swabs from the various places in the hospital and from healthy hospital staff. They obtained a percentage of 31.7% surprisingly all the staphylococcal isolates in their study were CNS. This study represents the magnitude of the hospital source from which infection can be transmitted to vulnerable patients through various invasive

procedures, if proper care and aseptic precautions are not taken.

Among the isolates in the present study 32% (Table 4) were from IV catheters Infections associated with intravascular devises is the present day problem, especially in intensive care units. As the procedure of IV cannulation is always performed as an emergency, there is often inadequate care for asepsis more over patients admitted inadequate care for asepsis. More over the patient admitted into the ICU is often severely ill and Immunocompromised. Due to this reason the coagulase negative staphylococci colonizing on the skin have an easy access to the device inside the body. The organisms can easily adhere to the device with the help of polysaccharide adhesin and produce slime with the help of which can survive on the device. Catheter associated bacterimia is common in the hospitalized patients. In the study of Goel et al 2006, 10.7% of the isolates were found from the catheter. The study of Goel is dated back to 2006 and the present study is under taken almost 2 years later, the high percentage of catheter isolates can be due to the generalized increase in the IV cannulation procedures, either for intravenous alimentation or for emergency parenteral infusion of drugs.

Maximum numbers of CNS isolates in the present study are from the wounds, 55.88% (Table 4). All samples collected from wounds are from inpatients and hence these isolates can be demarcated as hospital strains in the study of Goel et al 2006 38.2% of isolates were from wounds whereas(Gaikwad and Deodher1983) [6] obtained 76, 92% of their strains from wound exudates. Surgical sepsis is one of the commonest infections encountered in the hospital. The percentage incidence of surgical sepsis in any hospital is an indicator of quality of Medicare in the hospital, lesser the sepsis the better is the quality. Ideally a good hospital should have a percentage of less than four of surgical sepsis (Hospital infections control practices advisory committee 1999).

In the present study 14% of the CNS isolates are from blood (Table 4) Goel et al. (2006) obtained 14.7% of CNS isolates from blood, and (Gaikward and Deodhar 1983) [6] obtained 11.74% from blood. The overall rate of nosocomial blood stream infection has drastically increased since the past 2 decades due to reasons explained earlier (Forbes et al 2007) [7]. The incidence of blood stream infections with CNS is also on the rise due to spread of the organism from the IV cannula into the blood. If it is a case of burns or bed sores the associated blood stream the infections are all the more risky because of transmission of hospital residents through the colonized site. As very well understood hospital residents are multidrug resistant. Urinary isolates of CNS occupied 15% in the present study (Table 4) Goel et al 2006 have reported 28.4% of isolates from urine, and (Gaikwad and Deodhar 1983) [6], only 1 strain of CNS from urine. Urinary tract infections caused by CNS are very often associated with urinary catheterization . In the community E coli is the commonest urinary pathogen, while in the hospital CNS occupy the first place. The number of patients in hospitals and nursing homes with long term indwelling catheters continue to increase. With catheterization the colonizing populations of urine in the urethra get access into the bladder. The organisms rapidly colonize on the urinary catheter and produce cystitis. The infection develops more rapidly if the patient is non ambulatory. Diabetes mellitus is a predisposing factors and diabetic patients are more prone for developing compromised urinary system. Hospitalized diabetic patients are catheterized more often and are at higher risk of UTI with CNS (Mahon and Manusilis 2000) [8].

Only one isolate in the present study in the CSF (Table 4). CNS enters the central nervous system through intrathecal procedures like lumbar puncture. Some times when the resistance of the host is severely compromised like in the advance stage of HIV infection or late stage of terminal illness with nosocomial bacterimia, patients with corticosteroid therapy or chemotherapy are at a higher risk. The predominant species of CNS in present study is S epidermidis (Table 2), Of the 48 strains of S epidermidis isolated 19 were from catheter 12 from blood 8 each from urine and wounds and one from CSF (Table 9). S epidermidis is the most commonly encountered species among the CNS. Its prevalence as nosocomial pathogen is very much related to medical procedures and practices than the capacity of the organism to establish infection (Hebert et al 1988, Bailey and Scott 2000) [9] (Carloos et al 1991) [10] identified a endemic strain of S epidermidis in the hospital producing bacterimia in the neonatal intensive care unit. S epidermidis is a notorious slime producer and easily establishes biofilm on polymers within the biofilm the organism can limit the effectiveness of antibiotic therapy and multiplies further as reported by (Shoba et al 2005) [11] Staphylococcus epidermidis is prevalent in 49.23% of hospital sites including the skin of the health care providers. 14% of these strains are oxacillin resistant, thus S epidermidis can be termed as an important hospital pathogen and hospital infection control programs should include eradication of this organism from the hospital sites.

20% of CNS Isolates are identified as S

saprophyticus (Table 4,9). This organism unlike S epidermidis is more a member of the community. Urinary tract infections caused by Staphylococci in the community are mostly due to S saprophyticus. It is a common organism isolated from urine from community acquired urinary infections in young sexually active female. Establishment of S saprophyticus as pathogen requires repeated careful processing and quantitative urine cultures as the organism inhabits the normal urethra and perineal skin, it is likely to contaminate urine samples during collection.

The role of the microbiologist is crucial in this situation. In the present study 11% isolates of S saprophyticus are from the catheter, 8% from wounds and only 1% from urine. S saprophyticus like S epidermidis is also a normal flora of human skin and in addition it colonizes the mucosa of gentitourinary tract. Though it is an established cause of urinary tract infections, catheter related sepsis due to S saprophyticus is not well studied. S saprophyticus has high capacity to adhere and colonize on surfaces, but unlike S epidermidis it is a poor producer of slime (Kloos and Bannerman 1994) [12]. Hence, catheter colonization by S saprophyticus is not as dangerous as that with S epidermidis, since the former cannot produce a biofilm and cause bacterimia. S saprophyticus exhibits receptor mediated adherence to uroepithelial cells and production of urease by S saprophyticus helps in its survival in the urinary tract.

In the present study 15% of isolates are identified as S hemolyticus (Table 2) Among the 15 strains 11 are isolated from wound 3 from urine and 1 from IV cannulae (Table 9). S haemolyticus is also part of the human normal skin flora. It has been documented as a cause of nosocomial baterimia. wound and soft tissue infection, UTI, pediatric and neonatal bacterimias. Vancomycin resistance as been reported in this organism as well as multidrug resistance. The presence of multiple antibiotic resistant S hemolyticus in the hospital environment and transmission of resistant clones through the hands of health care workers havebeen documented by several investigators using molecular methods (Koneman 2006) [13] Compared to S epidermidis this organism colonizes in much fewer numbers and hence less commonly incorporated in clinical illness. The study of virulence factors in S hemolyticus has been futile thus it can be that the disease caused by this disease is milder.

S hominis isolates were 14% among the total CNS isolates in the present study (Table 4,9). Among the 14 strains 9 were from wound, 2 strains each from blood and urine and 1 from catheter. This species is a comensal in the skin of humans and has occasionally been isolated from infections as a low grade pathogen (Koneman 2006) [14]. Among the total CSF isolates it is infrequent and when it is isolated as a pathogen severity and extent of infection are less (Kloos and Bannerman 1994) [15]. However under antibiotic pressure it is known to develop resistant more easily and readily (Kloos and bannermann1994). Like many other coagulase-negative staphylococci, S. hominis may occasionally cause infection in patients whose immune system is compromised, for example by chemotherapy or predisposing illness.

S caprae strains occupied least important position 3% among the total CNS isolates in the present study (Table 2) among them 2 are from wound and 1 from urine (Table 9). S caprae is rarely cultured from clinical specimen when compared to S epidermidis it as been reported in association with bone and joint infection (Koneman 2006). Strains of S caprae isolated from humans known to contain a 5 gene Ica operon that coded for the gene products involved in biofilm formation. The gene products exhibit subtotal aminoacid identity with those of S epidermidis.

In the present study infections with CNS among males are more or less equal to that of females. There is no sexual predisposition (Table 6). The age group of more than 40 years had the highest incidence 44% CNS in present study (Table 7).

This is the age group which as most of the predisposing factors to CNS infections like lowered immune status, Diabetic Nephropathy, Hypertensive Nephropathy, malignancy, obstructive urinary tract diseases with frequent IV cannulation and urinary catheterization. Among the hospital isolates of CNS oxacillin resistance is 13.23%(Table 8), where as in community isolates it is much lower 9.37% (Table 9). Methicillin resistance among CNS clinical isolates were studied by Shoba et al 2005. They have reported 14% of methicillin resistance in hospital resident CNS flora. Similar studies have been reported by Vijayalakshmi et al (Shoba et al). Like in S. aureus methicillin resistance among strains of CNS is matter of serious concern.

Further studies are required to elaborate on multidrug resistance in hospital strains of CNS. The future microbiologists should aim at an efficient and patient team work to eradicate the hospital resident flora, more so the methicilline resistant flora. Vanocomycin resistance in the present study is 23.5% in hospital isolates of CNS and 18.75% in community isolates. Vancomycin is the drug of choice for treatment of methicillin resistant staphylococci and restricted and choicest use of this antibiotic can greatly reduce the risk of acquisition of resistance

by the organism. MIC of the clinical isolates for methiciline and vancomycin could not be studied for want of antibiotic in pure powder form in time.

Summary & Conclusions

1. In the present study CNS was the most predominant organism (105/50; 47.61%).
2. Most of CNS isolates were from the hospital infections (68%).
3. Coagulase negative Staphylococci have been increasingly gaining importance in hospital infections, compared to E. coli, Klebsiella, MRSA and Pseudomonas.
4. Majority of the isolates were from IV catheters (76.19%), stressing the need for more aseptic precautions in ICU settings.
5. Higher percentage of isolates from IV catheters points to increase in IV cannulation procedure, either by alimentation or by drug infusion.
6. Maximum numbers of CNS isolates were from wounds (38%).
7. Sizeable number (15%) CNS isolates are from urine samples. Improper precautions during catheterization and non ambulatory status of the patient contribute to this increase in UTI.
8. Considerable numbers of CNS isolates are from blood (14%). In case of burns or bed sores, the blood stream infections pose more threat as the organisms are usually multidrug resistant, being hospital resident.
9. Metabolic disorders like Diabetes mellitus compromise the urinary system predisposing to higher risk of UTI with CNS.
10. Immunosuppression either natural (HIV) or therapeutic (Steroids) can make patients more vulnerable to infections with CNS (Study with more number is required).
11. Most predominant species of CNS in this study is S. epidermidis, followed by S. saprophyticus.
12. Further studies with larger sample size are required to elaborate on multidrug resistance in hospital strains of CNS.

References

1. Pulverer, G., and J. Pillich. 1971. Pathogenic significance ofcoagulase-negative staphylococci, 1971. p.91-96. In M. Finland, W. Marget, and K. Bartmann (ed.), Bacterial infections: changes in their causative agents; trends and possible basis. Springer-1971.
2. Banerjee S.N.T.G Emori, D.H.Culver, R.P.Gayes W.R. Jarvis,T. Horan,J.R. Edwards J.S. Tolson,T. Henderson, W.J.Matrone and National nosocomial infection surveillance system 1991. Secular trends in nosocomial primary blood stream infections in united stats Am J. Med. 1991;91(Suppl.3B): 1980-1989; 865-895.
3. Koneman, Washington. winn. junior, Steph. Allen and Willium. Janda colour atlas and text book of Microbilogy. Ed., 2006;626-29.
4. Raksha R., Srinivasa H., Macaden R.S. Occurrence & Characterisation of Uropathogenic E. coli in urinary tract infections, Ind J of Medical Microbiology-2003 Oct:102-7.
5. Casewell and Philips, 1981 Casewell M.W., Philips I. Aspects of Plasmid mediated antibiotic resistance & epidemiology of Klebsiella spp. Am J Med 1981; 70:459-62.
6. Gaikwad SS, Deodhar LP. Study of coagulase-negative Staphylococci in clinical infections. J Postgrad Med 1983;29:162-4.
7. Forbes B.A., Sahm D.F, Weissfeld. A.S. Bailey & Scott's Diagnostic Microbiology Ed :12th, 2007;217:946.
8. Mohan V, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative staphylococci isolated from various clinical specimens. Indian J Med Microbiol 2002;20:45-6.
9. Hebert, G. A., R. C. Cooksey, N. C. Clark, B. C. Hill, W. R. Jarvis, and C. Thornsberry. 1988. Biotyping coagulase-negative staphylococci. J. Clin. Microbiol. 26:1950-1956.
10. Carlos, C. C., S. Ringertz, M. Rylander, P. Huovinen, and G. Faxelius. Nosocomial Staphylococcus epidermidis septicaemia among very low birth weight neonates in an intensive care unit. J. Hosp. Infect. 1991;19:201-207.
11. KL Shobha, PS Rao, J Thomas. Survey of Staphylococcus isolates among hospital personnel, environment and their antibiogram with special emphasis on methicillin resistance. Indian J Med Microbiol. 2005 Jul;23(3):186-8.
12. Kloos, W. E., K. H. Schleifer, and F. Gotz. 1991. The genus Staphylococcus, p. 1369 1420. In A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes. Springer-Verlag, NewYork. 1992.
13. Kloos, W. E., and D. W. Lambe, Jr. 1991. Staphylococcus, p. 222-237. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
14. Kloos, W. E., and J. F. Wolfshohl. Identification of Staphylococcus species with the API Staph-Ident system. J. Clin. Microbiol. 1982;16:509-516.

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Comparison of Different Phenotypic Tests for Detection of ESBL (Extended Spectrum Beta Lactamases) Producing Pseudomonas Aeruginosa

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Abstract

Background: P. aeruginosa is the most common nosocomial pathogen encountered and a known organism causing high mortality and morbidity. Due to indiscriminate antibiotic use, resistance is very commonly known in them and ESBL enzyme production being the most predominant one. *Material and Methods*: A total of Ninty isolates of P. aeruginosa were tested for the presence of ESBL enzyme by both disc diffusion and double disc synergy test. Antibiotic sensitivity pattern of ESBL-positive P. aeruginosa was determined. *Results*: Of the 1200 pus samples screened, 90 isolates of pseudomonas were tested for ESBL production. Of the 90 P.aeruginosa isolates, 57 (63%) were sensitive to 3GC and 33 (37%) were resistant.Of the 33 P.aeruginosa resistant to ceftazidime, DDT detected 9 (27%) of ESBL producers and DDST detected 17 (51%). And 17 (51%) did not show ESBL production by either of the methods used in the study. All the ESBL-positive P. aeruginosa were multi-drug resistant, with 100% sensitivity to imipenem; followed by ofloxacin (70%). *Conclusion*: From this study, we conclude that DDST proved to be better method than DDT to detect ESBL producing P. aeruginosa. However in the absence of any CLSI guidelines for detection of ESBL in Non-fermenters, we reframe from commenting on specificities of either of tests. There is a strong need for standardization / CLSI guidelines for detection.

Keywords: Third Generation Cephalosporins; Double Disk Synergy Test; Disc Diffusion Test; P. Aeruginosa.

Introduction

Pseudomonas aeruginosa is one of the most prevalent opportunistic human pathogen and the most common gram-negative bacteria causing nosocomial infections belonging to ESKAPE group of pathogens (Enterococcus faecium, Staphylococcus aureus, Klebseilla pneumonia, acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species). A major challenge has aroused in treatment of Pseudomonas aeruginosa infections exhibiting high level resistance to all antibiotic classes, not only due to innate nature but due to their additional acquiring through plasmids [1]. ESBL mediated resistance is one of the important emerging resistance mechanisms. ESBL enzyme encoding genes SHV-2a and TEM-42 are responsible for the same [2,3].

At present Clinical Laboratory Standards Institute (CLSI) guidelines do not describe any methods for detection of these enzymes in P. aeruginosa [4].

Hence this study was conducted to know the prevalence and to compare the different phenotypic tests for detection of ESBL (Extended Spectrum Beta lactamases) producing P. aeruginosa isolates from pus samples

Objective/Aim

Present study was conducted to compare the different phenotypic tests for early and accurate detection of ESBL (Extended Spectrum Beta lactamases) producing P. aeruginosa isolates from pus samples.

Material and Methods

A total of 1200 pus samples were screened in one year which were received at the Department of Microbiology, J. N. Medical College, KLE University, from hospitalized patients of K.L.E.'S DR. Prabhakar Kore's Charitable Hospital and MRC, Belagavi.

Only those isolates of P. aeruginosa obtained from pus samples as pure and predominant growth were included in the study.

Based on colony morphology and biochemical tests, organisms were identified. Using disc diffusion method, sensitivity of the isolates to the third-generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, 30µg each) was determined using P. aeruginosa ATCC 27853 as control strain. Results were interpreted according to the CLSI guidelines, which suggest a diameter of inhibition zone > 22 mm for ceftazidime, > 27 mm for cefotaxime and >25 mm for ceftriaxone as susceptible [5].

Only those isolates showing resistance to third generation cephalosporins were tested for ESBL production by the following two methods .

a) Disc diffusion test

b) Double Disc Synergy Test

- *Disc Diffusion Test* [6]:

Ceftazidime (30mcg) & with Ceftazidime/ Clavulanic acid (30mcg/10mcg) discs were placed on Mueller Hinton agar (MHA, Hi-Media) inoculated with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture and was incubated overnight at 37^{0}C.

An increase in the zone diameter by > 5mm of Ceftazidime versus its zone when tested in combination with Clavulanic acid was considered as an ESBL producer.

- *Double Disc Synergy Test [7]:*

30mcg disc of each third generation cephalosporin antibiotics: Cefotaxime, Ceftriazone and Ceftazidime, are placed on Mueller Hinton agar (MHA, Hi-Media) inoculated with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture at distance of 15mm center to center from Augmentin disc (Amoxicillin/Clavulanic acid- 20mcg/10mcg) and was incubated overnight at 37^{0}C.

Increase in the inhibition zone of any one of the three third generation antibiotic disc towards augment disc was considered as an ESBL producer.

In both the methods, increase in zone size occurs because the clavulanic acid present in the amoxyclav disc inactivates the ESBL produced by the test organism.

Results

Out of 1200 pus sample screened, 90 P. aeruginosa isolates were isolated.

Of the 90 P.aeruginosa isolates, 57 (63%) were sensitive to 3GC and 33 (37%) were resistant.

Of the 33 P.aeruginosa resistant to ceftazidime, DDT detected 9 (27%) of ESBL producers and DDST detected 17 (51%). And 17 (51%) did not show ESBL production by either of the methods used in the study.

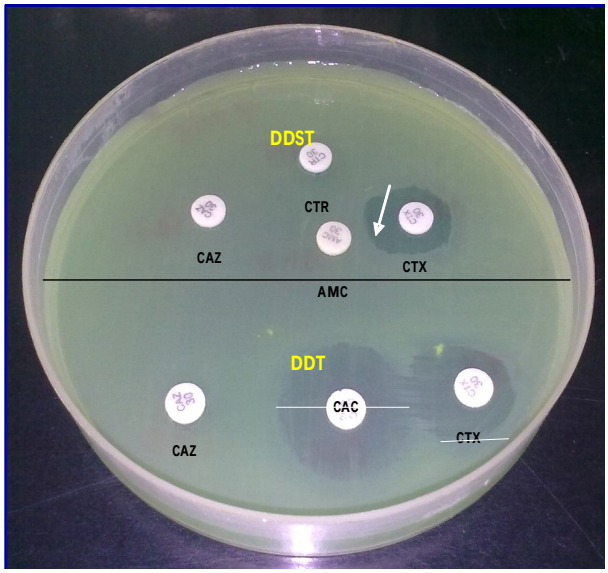
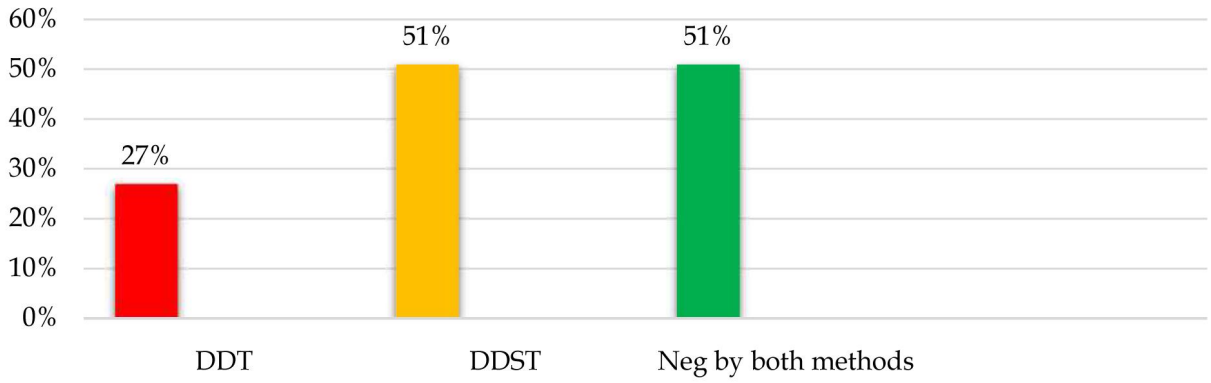


Fig. 1: Extended-spectrum-â-lactamase(ESBL) producing *P.aeruginosa* detection by Double disk synergy test(DDST)& Disc diffusion test (DDT):
Interpretation of DDST: Increase in inhibitory zone around CTX antibiotic disc towards Augmentin disc.
Interpretation of DDT: Increase in zone diameter of Ceftazidime by > 5mm.



Graph 1: Percentage of ESBL producing *p.Aeruginosa* Detected by different methods

All the ESBL-positive P. aeruginosa were multi-drug resistant (drug resistance to more than three drugs). Maximum sensitivity (100%) was seen with imipenem; followed by ofloxacin, which showed good sensitivity (70%). The least effective drugs were cephalothin, cefamandole, azlocillin, ticarcillin, ticarcillin/clavulanate.

Conclusion

In the present study 36.6% (33/90) P.aeruginosa were resistant to Ceftazidime which is similar to the study done by Aggarwal et al [7], at Haryana showing 20.27% of Cefazidime resistant P.aeruginosa. Another study done by Singh et al [8], at Mysore also showed 27.2% P.aeruginosa resistant to Cefazidime, 20.9% by a study done by Zahra et al [9], West Bengal and 20.27% by a study by Wayne et al [10].

Studies in some places like in Nagpur, the figures of ESBL producers were 50% [11] and another comparatively recent study in 2005, from New Delhi, showed 68.78% of the strains of gram negative bacteria to be ESBL producers [12], which is high compared to our study. And studies in few other places like in Varanasi, Upadhyay S et al showed the prevalence of ESBL producing P.aeruginosa was 3.3%[13] and Rodrigues C et al, in their study showed 5.9% of P.aeruginosa isolates harbored ESBLs in Mumbai [14], which is less in comparison to our study.

This variation in the prevalence of ESBL producing P.aeruginosa in different places/studies could be due to the variation in sample size studied or due to their differences in hygienic practices.

In our study of the 33 P.aeruginosa resistant to Ceftazidime processed for ESBL detection, Double Disc synergy test detected 17(51%) compared to Disc diffusion test which detected 9(27%) of ESBL producing P.aeruginosa and thus DDST proved to be better method than DDT to detect ESBL producers.

A similar study by Umadevi S et al [15], in which the the two conventional methods DDST and DDT were compared. They found, no significant differences between the ESBL detection rates by two conventional methods in P. aeruginosa. Their failure to detect the better performance of the double disk synergy test as compared to the disk diffusion test for the detection of ESBL production among the Pseudomonas aeruginosa isolates could be due to the relatively small number of isolates which were tested in their study (27 isolates).

A study done by Jiang X et al, to detect ESBL producing P.aeruginosa from 75 isolates showed that there were no ESBL false positive detected in the ESBL-screening methods like DDST and Combined Disc test when compared to IEF (isoelectric focusing electrophoresis), PCR, and PCR product sequencing. And hence found the conventional methods to be more cost effective, easy to perform in routine clinical laboratory and are as sensitive as molecular techniques like IEF and PCR [16].

In a study by Shukla et al, they found DDT to be more sensitive for detecting ESBL producers than the DDST. And the reason coated was the problem of optimal disc space and correct storage of the Clavulanate containing disc [17].

The 17 (51%) Ceftazidime resistant P.aeruginosa which gave negative results both methods used to detect ESBL producing may have other mechanism of resistance such as impermeability of outer membrane and or active efflux mechanism or may be due to masking effect of presence of AmpC or due to MBL production by the organism.

The main limitation of our studies were, due to the absence of any standard methods to detect ESBL in non-fermenters, it is difficult to comment on true or false ESBL producers, MIC reduction would be a better method to know the drug susceptibility, but it is a cumbersome, laborious method and PCR could have been an additional investigation to detect the genes responsible for resistant, but has the disadvantage of its high cost.

This early and accurate detection of ESBL producing P.aeruginosa has helped the doctors to treat the patients early with appropriate antibiotics, thereby improving the patient outcome and decreased the morbidity and mortality.

However in the absence of any CLSI guidelines for detection of ESBL in Non-fermenters, we reframe from commenting on specificities of either of tests. There is a strong need for standardization / CLSI guidelines for detection.

References

1. Shahid M, Malik A, Sheeba. Multidrug resistant *Pseudomonas aeruginosa* strains harbouring R-plasmids and Amp C β-lactamases isolated from hospitalized burn patients in tertiary care hospital of North India. FEMS Lett 2003;228:181-6.
2. Naas T, Phiippon L, Poirel L, Ronco E, Nordmann P. An SHV-derived extended-spectrum β-lactamase in *Pseudomonas aeruginosa* .Antimicrob Agents Chemother 1999;43:1281-4.
3. Mugnier P, Dubrous P, CasinI, Arlet G, Collatz E. A TEM-derived extended-spectrum β-lactamase in *Pseudomonas aeruginosa* .Antimicrob Agents Chemother 1996;40:2488-93.
4. Clinical laboratory standards institute. Performance standards for antimicrobial susceptibility testing. Sixteenth international supplement. CLSI document M100 - S 16, Wayne PA: 2007.
5. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests, 7th ed. Approved standard. NCCLS document M2-A7, Vol. 20 No. 1; Wayne PA: January 2000.
6. Jayakumar S, Appalaraju B. Prevalence of multi and pan drug resistant *Pseudomonas aeruginosa* with respect to ESBL and MBL in a tertiary care hospital. Indian J PatholMicrobiol. 2007;50(4):922-5.
7. Aggarwal R, Chaudhary U, Bala K. Detection of extended-spectrum beta-lactamase in *Pseudomonas aeruginosa*. Indian J PatholMicrobiol. 2008;51(2):222-4.
8. Singh M, Pal KN, Banerjee M, Sarkar **S**and Gupta MS.Surveillance on Extended Spectrum β-lactamase and AmpC β-lactamase producing gram negative isolates from nosocomial infections. Arch of clin Microbiol. 2012;3:1-7.
9. Tavajjohi Z, Moniri R and Khorshidi A. Detection and characterization of multidrug resistance and extended-spectrum-beta-lactamase-producing (ESBLS) *Pseudomonas aeruginosa* isolates in teaching hospital.African Journal of Microbiology Research. 2011;5(20):3223-3228.
10. Wayne PA (2010) Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement M100-S20.
11. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res. 2004;12(5):553–6.
12. Mohanty S, Singhal R, Sood S, Dhawan B, Das BK, Kapil A. Comparative in vitro activity of beta-lactam / beta-lactamase inhibitor combinations against Gram negative bacteria. Indian J Med Res. 2005;122(3):425–8.
13. Upadhyay S, Sen MR and Bhattacharjee A. Presence of different beta-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC beta-lactamase enzyme. J Infect Dev Ctries. 2010;4(4): 239-242.
14. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R and Mehta A.Detection of Beta-lactamases in nosocomial Gram negative clinical isolates. Ind J Med Microbiol. 2004;22(4):247-250.
15. Umadevi S. Prevalence and antimicrobial susceptibility pattern of ESBL producing Gram Negative Bacilli. Journal of Clinical and Diagnostic Research 2011;5(2):236-239.
16. Jiang X, Zhang Z, Li M, Zhou D, Ruan F, Lu Y. Detection of extended-spectrum beta-lactamases in clinical isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2006;50(9):2990-5.
17. Shukla I, Tiwari R and Agarwal M. Prevalence of ESBL producing Klebsiellapneumoniae in a tertiary care hospital. Ind J Med Microb. 2004;22(2):87-91.

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Study of Automated Urine Analyser Iris IQ 200 in Predicting Urine Cultures

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Abstract

Background: Many automated urine analysers like IRIS IQ 200 and IChem Velocity (Beckman coulter) performing automated biochemical and microscopic examination are now available to improve the accuracy and productivity of urine examination and minimize the inter observer variability. It has been specially useful in predicting the growth of microorganisms in urine culture thereby reducing the time from diagnosis to initiation of treatment. *Objective:* The present study was undertaken to evaluate the role of fully automated Iris IQ200 and IChem velocity workstation (Beckman Coulter) in predicting urine infections and those needing culture. *Materials and Methods:* A total of 252 patients coming to our diagnostic centre for urine examination from January to August 2017 were included in the study.Urine was inoculated on blood and McConkey Agar plates with a 0.01 ml calibrated loop and after incubating at 37^0C for 24 hours, bacterial growth and colony identification was carried out by standard protocol. A significant growth was considered with a colony count of 10^4 CFU / ml. *Results*: Leucocyte esterase was positive in 82 (32.5%) cases, nitrite in 23 (9.12%), bacteria in 72 (28.5%) and bacterial growth in culture in 99 (39.2%) patients. Out of these insignificant growth was seen in 22 (22.23%) and significant growth in 77 (77.77%). Out of 99 cases which showed bacterial growth in culture, E.coli was grown in 70 cases with 56 patients showing significant colony count. Klebsiella sps was grown in 21 cases, Enterococcus and Enterobacter sps in three cases, Pseudomonas in two cases. *Conclusion:* Automated urine analysers are good predictors of urine cultures and UTI and have enhanced accuracy due to reporting of leucocyte esterase, nitrite, bacteria and all small particles.

Keywords: iQ 200; iChem Velocity; ASP; Leukocyte Esterase; Nitrite.

Introduction

Urinary tract infection is one of the most frequently encountered medical problem all over the world [1]. It ranges from asymptomatic bacteriuria to severe septic infections. Females are more prone to UTI because of their short urethra as compared to men. UTI are mostly diagnosed by simple and rapid routine urine analysis which includes both biochemical tests for presence of blood and protein as well as microscopic examination for presence of pus cells, RBCs, bacteria and casts [2]. Although simple and affordable, the routine urine examination is time consuming and labour intensive and has a lot of inter observer variation due to subjective interpretation.

The European urinanalysis guidelines recommends two steps for urine analysis [3]. In the first step, dipstick is used to analyse the biochemical parameters. If all the parameters are negative, no further microscopy is needed. If dipstick is positive for Hb, protein, leukocytes and nitrite, microscopy is done. But this has a fair chance of missing infections [4,5,6,7]. Many automated urine analysers like IRIS IQ 200 and IChem Velocity (Beckman coulter) performing automated biochemical and microscopic examination are now available to improve the accuracy and productivity of urine examination and minimize the inter observer variability. It has been specially useful in predicting the growth of microorganisms in urine culture thereby reducing the time from diagnosis to initiation of treatment. Currently, there is no standard reference method available for urine microscopy which can provide correct identification of formed elements in urine microscopy.

Manual microscopic examination requires trained Staff with experience. To overcome these shortcomings, automated urine analysers were developed for high volume laboratories to provide both standardisation and improve the turnaround time [8,9].

The present study was undertaken to evaluate the role of fully automated Iris IQ200 and IChem velocity workstation (Beckman Coulter) in predicting urine infections and those needing culture.

Material and Methods

A total of 252 patients coming to our diagnostic centre for urine examination from January to August 2017 were included in the study in all ages and both the sexes. Mid stream Urine samples were collected according to standard protocol in wide mouth, leak proof sterile containers. Each sample was subjected to urine analysis within one hour of collection. Urine was inoculated on blood and McConkey Agar plates with a 0.01 ml calibrated loop and after incubating at 37°C for 24 hours, bacterial growth and colony identification was carried out by standard protocol. A significant growth was considered with a colony count of 10^4 CFU/ml. Routine urine analysis was carried out on IRIS iQ 200 and iChem Velocity (Beckman Coulter) using unspun urine specimens for presence of WBCs, leukocyte esterase, nitrite, bacteria and all small particles (ASP).

iQ 200 was calibrated by iQ focus , iQ negative and iQ positive controls (IRIS diagnostics)every day before running the urine samples.

Patients on antibiotic therapy and cathetrised patients were excluded from the study.

Results

A total of 252 urine specimens were analysed for the presence of leucocyte esterase, nitrite, WBCs, bacteria and ASP on iQ 200 in both sexes and all age groups. The patients were divided into less than 20, 21-40, 41-60, 61-80 and more than 80 years of age in both sexes. There were 95 (37.6%) males and 157 (62.4%) females. The male to female ratio was 0.60:1. (Table 1). Bacterial growth was observed in 99 (39.2%) cases while in 153 (60.8%) cases the urine was sterile. Maximum cases 72 (28.57%) were in 21-40 years of age with 54 females and 18 males, followed by 69 patients (27.3%) in 41-60 years of age with 27 males and 42 females. In 0-20 years of age, there were 22.22% cases, 19.84% in 61-80 and least (1.98%) above 80 years of age (Table 1).

WBCs; Leukocyte Esterase; Nitrite and Bacteria

In presence of 0-5 WBCs/hpf 22 cultures were positive for bacterial growth. Out of these, 13 showed insignificant bacterial growth (< 10^4 CFU / ml) and 9 cases showed significant growth(>10^4 CFU / ml).

When pus cells were between 6-10 / Hpf , Leukocyte esterase was positive in 5 cases. 5 had bacterial growth in culture out of which 2 had significant growth.

When WBCs were 11-20 / hpf, leukocyte esterase was present in 12 cases, nitrite in one and presence of bacteria in 6 cases, out of which 5 showed bacterial growth in culture out of which 4 had significant colony count.

When WBCs were between 21-30 / hpf, leukocyte esterase was present in 10 cases, 2 had nitrite positivity and presence of bacteria in 9 cases. Out of these 21 cases showed bacterial growth in culture with significant colony count in 17 patients. In 12 cases bacteria was not detected by iQ200 but had culture positivity.

When WBCs were more than 30/hpf, it was observed that 57 cases had leukocyte esterase positivity, 20 had nitrite present in the urine and all the 57 ases had bacteria in their urine, detected by iQ200. Out of these 57 cases, 46 showed significant bacterial growth in culture (Table 2).

Leucocyte esterase was positive in 82 (32.5%) cases, nitrite in 23(9.12%) , bacteria in 72(28.5%) and bacterial growth in culture in 99(39.2%)patients. Out of these

insignificant growth was seen in 22 (22.23%) and significant growth in 77 (77.77%). Out of 99 cases which showed bacterial growth in culture, E.coli was grown in 70 cases with 56 patients showing significant colony count. Klebsiella sps was grown in 21 cases, Enterococcus and Enterobacter sps in three cases, Pseudomonas in two cases. (Table 3).

Table 1:

Showing Demographic Data of Patients				
Age in years	**Male**	**Female**	**Total**	**Percentage**
0 to 20	18	38	56	22.22
21 to 40	18	54	72	28.57
41 to 60	27	42	69	27.3
61 to 80	29	21	50	19.84
>80	3	2	5	1.98
TOTAL	95	157	252	
percentage	37.6	62.4		

Table 2:

showing relative distribution of patients w.r.t. biochemical parameters						
Pus cell	**Leukocyte Esterase**	**Nitrite**	**Bacteria**	**Culuture Positive**	**Colony count $>10^4$/ml**	**$<10^4$/ml**
0 to 5	1			22	9	13
6 to 10	2			5	2	3
11 to 20	12	1	6	5	4	1
21 to 30	10	2	9	21	17	4
>30	57	20	57	46	45	1
total	82	23	72	99	77	22
percentage	32.5	9.12	28.5	39.2	77.77	22.23

Table 3:

Showing Bacterial Growth			
Colony	**$<10^4$/ml**	**$>10^4$/ml**	**Total**
Kebsiella	7	14	21
Enterococcus	1	2	3
Pseudomonas	0	2	2
Enterobater	0	3	3
E.Coli	14	56	70
Total	22	77	99

Fig. 1:

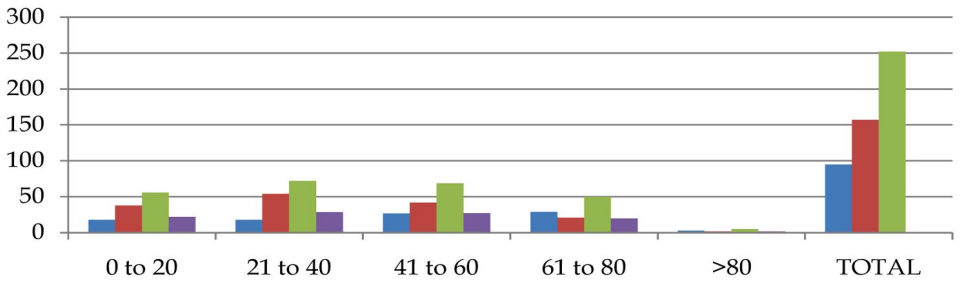
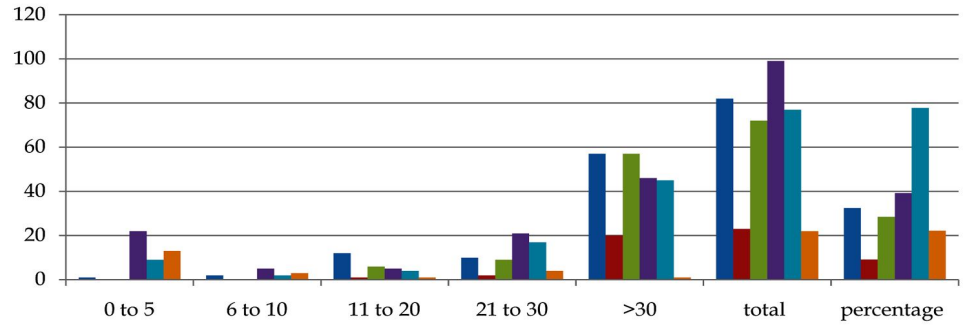


Fig. 2:



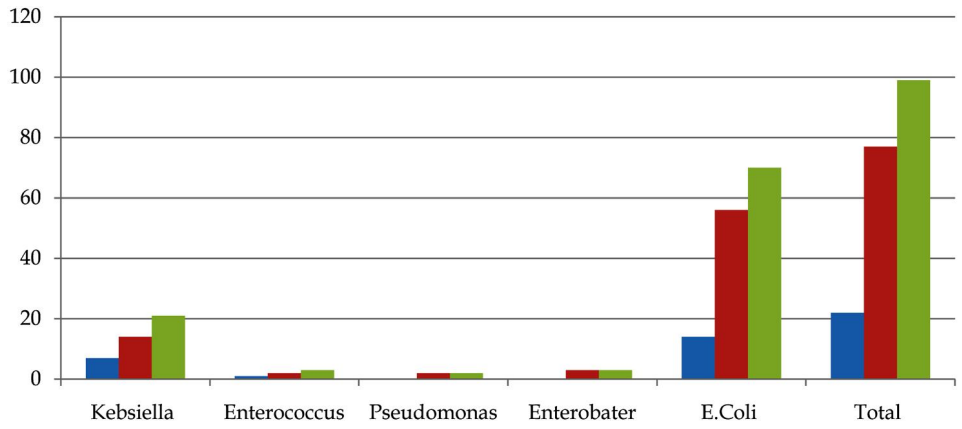


Fig. 3:

Discussion

Iris IQ200 and IChem velocity (Beckman Coulter) have been introduced in medical laboratories in recent times for complete examination of urine which has resulted in improved standardization of urine analysis and more efficient working. The main purpose of automated urine analysers is to minimize the number of urine samples subjected to culture by screening normal from abnormal specimens. This in turn can reduce the financial burden and labour cost involved in culture and also minimize the turnaround time significantly.

The integrated IRIS iQ 200 and iChem Velocity (Beckman Coulter)automated urine analyser works on the principle of flow digital image capture technology and uses an autoparticle recognition software (APR) to identify particles in urine. The urine is aspirated in the analyser which is then focused hydrodynamically between the two layers of suspending fluid(planar flow) forcing the particles to orient in a single plane which faces a microscopic objective lens and a digital camera which captures at least 500 different fields for particles. The APR software then classifies these particles into WBCs, RBCs, squamous cells, casts, crystals, WBC clumps, bacteria, yeast cells, mucus, sperms etc. These images are then screened and saved for reporting. Chemical analysis is carried out by relectance spectroscopy which includes Ph, Specific gravity,nitrite and leucocyte esterase [10].

In our study we found a good correlation between abnormal urines with respect to presence of WBCs, leukocyte esterase,nitrite and presence of bacteria. Base on these findings, the instrument flags those specimens which require culture. We observed that as the number of WBCs increased, bacteria and leukocyte esterase showed positivity in increasing numbers. When WBCs were more than 30 /hpf, bacteria were present in all the 57 cases and culture showed significant bacterial growth in 46 cases (80.7%) based on the flagging by the instrument.

Sturenberg et al in 2014, analysed 963 urine specimens on the iQ200 system and concluded that when WBCs were more than 25/ul and bacterial count was more than 5 bacteria/ul ,the sensitivity was 98.9%.They concluded that approximately 30.4% to 35.9%samples can be excluded from being cultured by iQ200 microscopy [11]. Broeren MA et al observed that culture savings effect was greatly reduced to 20% of which 14%were false negative results as compared to 52% by using a cut off value of >/=10^5CFU/ ml [12]. Ami P. Shah et al in their study observed that sensitivity for pyuria was 84.5% by IRIS iQ200 [13]. They analysed 703 urine spevcimens and concluded that the sensitivity and PPV for urine culture was 79.5% and 37.5% respectively. In similar studies carried out by Noyan et al in 2014, it was observed that there was a good correlation between iQ200 results and culture positivity when WBCs, leukocyte esterase, nitrite and all small particles were taken into consideration [14]. On the other hand, Akin et al did not find a significant correlation between iQ200 and culture results [15]. Parta et al also found that ASP did not increase specificity ,sensitivity and NPV of bacterial cultures [16].

Capelletti et al observed that ASP and leukocytes are efficient tools for screening urine specimens for bacterial cultures [17].

Conclusion

The results of our study show a good correlation between iQ 200 and culture results. The main purpose of using automated urine analysers is to reduce the number of samples subjected to urine culture.These analysers decrease the turnaround time and financial

burden on the patients by avoiding unnecessary urine cultures . So, it can be concluded that automated urine analysers are good predictors of urine cultures and UTI and have enhanced accuracy due to reporting of leucocyte esterase, nitrite, bacteria and all small particles.

Conflict of Interest: None

References

1. Tanagho E.A., McAninch J.W. Smith's general urology. Sixth edition. Lange Medical Books, NY, 2004.
2. Delanghe J.R., Kouri T.T., Huber A.R., et al. The role of automated urine particle flow cytometry in clinical practice. Clin Chim Acta 2000;301:1-18.
3. European Urinalysis Guidelines Scand. J Clin. Lab. Investig., 2000;60(Suppl 231):S1-S86.
4. T. Shaw Jr, S.Y. Poon, E.T. Wong" Routine urinalysis". Is the dipstick enough? J Am. Med. Assoc., 1985; 253:1596-1600.
5. P. Laine, E. Toivonen, K. Eklund, U. Hohenthal, S. Siren, T. Maki Rapid dipstick urinalysis in the internal medicine clinic: what is missed?J. Intern. ed., 1997;242:271-273.
6. W.L. Deville, J.C. Yzermans, N.P. van Duijn, P.D. Bezemer, D.A. van der Windt, L.M. BouterThe urine dipstick test useful to rule out infections. A meta-analysis of the accuracy BMC Urol., 2004;4:4.
7. R. Gangaram, P.J. Ojwang, J. Moodley, D. Maharaj The accuracy of urine dipsticks as a screening test for proteinuria in hypertensive disorders of pregnancy Hypertens. Pregnancy, 2005;24:117-123.
8. Lamchiagdhase, K. Preechaborisutkul, P. Lomsomboon, P. Srisuchart, P. Tantiniti, N. Khan-u-ra, B. PreechaborisutkulUrine sediment examination: a comparison between the manual method and the iQ200 automated microscopy analyzer Clin. Chim. Acta, 2005;358:167-174.
9. R.J. Elin, J.M. Hosseini, J. Kestner, M. Rawe, M. Ruddel, N.N. NishiComparison of automated and manual methods for urinalysis. Am. J Clin. Pathol., 1986;86:731-737.
10. Stürenburg E, Kramer J, Schön G, Cachovan G, Sobottka I. Detection of significant bacteriuria by use of the iQ200 automated urine microscope. J Clin Microbiol. 2014;52(8):2855-60.
11. Enno Stürenburg, Jan Kramer, Gerhard Schön, Georg Cachovan, andIngo Sobottka Detection of Significant Bacteriuria by Use of the iQ200 Automated Urine MicroscopeJ Clin Microbiol. 2014 Aug;52(8): 2855–2860.
12. Broeren MA, Bahçeci S, Vader HL, Arents NL. Screening for urinary tract infection with the Sysmex UF-1000i urine flow cytometer. J. Clin. Microbiol. 2011;49:1025–1029.
13. Ami P. Shah, Benjamin T. Enhanced vs automated urinanalysis for screening of urinary tract infections in children in the emergency department, Pediatr Infect Dis J 2014 Mar;33(3):272-5.
14. Tevfik Noyan , Özgül Gözlükaya , Sema Nur Ayyildiz, Yeliz Çetinkol , Fazilet Özenç Çakir , Soner Çankaya Are All Small Particles Parameters in the iQ200 Auto Particle Recognition Software Have Any Benefit on Reduce the Urine Culture Number? Journal of Chemistry and Biochemistry December 2014;2(2).169-177.
15. Akýn OK, Serdar MA, Cýzmecý Z, Genc O. Evaluation of specimens in which the urine sediment analysis was conducted by full-automatic systems and a manual method together with urine culture results. Afr J Microbiol Res 2011;5(15):2145-49.
16. Parta M, Hudson BY , Le TP, Ittmann M, Musher DM, Stager C. IRIS iQ200 workstation as a screen for performing urine culture. Diagn Microbiol Infect Dis 2013;75(1):5–8.
17. Cappelletti P, Poz D, Metus P. Performance of IRIS iQ200 in urinary tract infections (UTIs) screening. Clin Chem 2009;55(6):A27.

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To Characterize & Determine the Virulence Factors of *Staphylococcus aureus* in a Tertiary Care Hospital

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Abstract

Context: *Staphylococcus* is a gram-positive, non-motile, nonsporing bacteria that include different opportunistic pathogenic species, responsible for human and animal infections. *Aims*: To isolate and identify *Staphylococcus aureus* from different human clinical samples and to determine various virulence factors of these isolates. *Settings and Design:* Prospective study conducted in Dept of Microbiology, SKIMS J&K. *Methods and Material:* Various clinical samples eg pus, blood, sputum etc received in the Microbiology laboratory were processed for isolation of Gram positive cocci. Identification of *Staphylococcus aureus* was done by standard microbiological methods and various virulence factors were determined. *Statistical Analysis Used:* Descriptive statistics (frequency and percentage) was used. *Results:* A total of 217 strains of *Staphylococcus aureus* were isolated from 1100 clinical specimens. All the isolates had ability to produce free and bound coagulase enzyme. Out of 217 strains, 42 (19.35%)strains showed only α hemolysin production, 84 (38.70%) strains showed only β hemolysin production and rest 91(41.93%) strains showed both α and β hemolysin production. Among all the strains, 203 (93.54%) were positive for producing biofilm and rest of 13 were negative for producing biofilm. Out of 217 strains of S.aureus only 49 (22.58%) strains were able to produce the staphyloxanthin pigment and rest 168 (77.41%) were found to be negative. All strains of *S.aureus* were sensitive to Linezolid, Teicoplanin and vancomycin. On the other hand strains showed high resistance towards Cefoxitin (65.89%). *Conclusions:* Prevalence of methicillin rsistance was high in IPD setting (65.89%) and production of various virulence factors like coagulase, hemolysin production, biofilm production and staphyloxanthin pigment production was more common in MRSA than in MSSA thus suggesting that infection control policies should be adhered positively.

Keywords: *Staphylococcus Aureus*; Prevelance; Virulence Factors.

Introduction

Staphylococcus is a gram-positive, non-motile, nonsporing ubiquitous bacteria that include different opportunistic pathogenic species, responsible for human and animal infections. They are facultative anaerobes. They appear as grape like clusters when viewed under the microscope and has large, round, golden- yellow colonies, often with hemolysis when

grown on blood agar plates [1]. On the basis of the ability to clot blood plasma, they are divided into two groups: *coagulase negative staphylococci (CoNS),* and *coagulase positive staphylococci (CoPS*) that include *Staphylococcus aureus* specie.

Staphylococcus aureus is one of the major pathogens of humans; it causes various suppurative diseases, food poisoning, pneumonia, and toxic shock syndrome [2,3]. About 20% of the population is always colonized with S. aureus, 60% are intermittent carriers, and 20% never carry the organism. In some, but not all, developed countries, many nosocomial infections are caused by *S. aureus* strains that are multiple resistant to antibiotics—known as Methicillin resistant *Staphylococcus aureus* (MRSA) [4-5]. Methicillin resistance is determined by the presence of a penicillin-binding protein with decreased affinity to penicillin. The mecA gene encodes this protein and is located on the staphylococcal cassette chromosome mec (SCCmec) [6-11].

Virulence of S.aureus

It is well known that *S. aureus* produces many virulence factors, such as hemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins, and immune-modulatory factors [2,12-14]. The expression of these factors is tightly regulated during growth. The relative importance of host factors versus bacterial virulence determinants in disease pathogenesis is unknown, but it is widely held that bacterial factors including toxins, cell wall-associated adhesins, and secreted exoproteins are involved in the process [15]. Thus, the pathogenicity of both *S. aureus* and CoNS, comes from their production of an impressive repertoire of virulence factors [16] that includes: surface proteins, that promote colonization of hosts tissues; invasions, that promote bacterial spread in tissue[17]; surface factors, that inhibit phagocyte engulfment [18].

Thus this study was undertaken to isolate *Staphylococcus aureus* from various clinical isolates and to determine various virulence factors of these isolates.

Subjects and Methods

This study was done in the Department of Microbiology SKIMS J&K in 2016 Sample Collection: Various clinical samples eg pus, wound swabs, urine, sputum, blood, various body fluids, tissue and tracheal tips received in the Microbiology laboratory were processed for isolation of Gram Positive Cocci. Identification of *Staphylococcus aureus* was done by standard microbiological methods and various virulence factors were determined such as coagulase activity, hemolysin production, biofilm production and staphyloxanthin pigment production.

Coagulase Activity Test

Coagulase test is based on the ability of *S. aureus* to produce a protein product called coagulase. There are two types of coagulase; Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. This type can be detected by the rapid slide Coagulase (SC) technique [19]. This test was performed on a clean slide using a sterile dropper; a small drop of saline was placed on the appropriate end of the slide as a control then a small drop of human plasma was placed on the opposite end of the slide with a sterilized loop. Cells were collected from one colony and were emulsified in the saline and then a drop of plasma was added, clumping was checked within 10 seconds of adding the bacterial cells to the plasma. On the other hand, the control drop, saline, showed no clumping of bacterial cells. The clumping will become more visible if the slide is rocked gently. The second type of coagulase is free coagulase which converts fibrinogen to fibrin by activating a coagulase reacting factor present in plasma which can be detected by the clumping of bacterial cells in the tube coagulase (TC) technique. Free coagulase activity was determined by the method described by Quinn et al [20] several colonies of each organism were mixed with 0.5 ml of citrated human plasma in a sterile test tube. The tube was incubated at 37°C for 4 hrs and examined after 4 and then kept at room temperature and examined at 24 h. Clot formation at either reading was recorded as positive.

Hemolysin Production

Alpha-hemolysin was evaluated on TSA supplemented with 5% washed human erythrocytes. The plates were incubated for 24 h at 37°C, when positive samples showed a wide zone of complete hemolysis with blurred edges. Beta-hemolysin was evaluated by plating strains on 5% sheep blood TSA. The plates were incubated at 37°C for 24 h and then overnight at 4°C, positive strains showed a wide zone of incomplete hemolysis with sharp edges [21-22].

Biofilm Formation

Quantitative determination was carried out by the

Micro plate method (MP) proposed by Pfaller et al [23]. Using tissue culture plates of 96 flat bottomed wells. Each well was filled with 0.2 ml of 10 [5] CFU / ml of a bacterial suspension in TSB. After 24h incubation in aerobic condition at 37°C, the contents were aspirated and plates were washed twice with phosphate buffered saline (PBS, pH: 7.2). The wells were stained with 0.1% crystal violet for 2 min. The plates were read in Micro plate reader (BioRad iMark™) to 492 nm. Sterile TSB was used as negative control. All the experiments were repeated at least twice and the values of optical density (OD) were then averaged. A three grade scale was used to evaluate the strains slime producing ability by comparing with OD of negative control or cut off (ODc): nobiofilm producer or (-):= ODc; (Weak): = 2x ODc; (Moderate): 2x ODc < ~ = 4x ODc; (Strong): > 4x ODc.

Staphyloxanthin Assay

The bright golden coloration of this virulence factor facilitates the virulence screening by the simple observation of color [24]. Also, a quantitative carotenoid assay method was adapted from the previous method [25]. In brief, cells were re-inoculated at 1:100 dilutions in TSB medium and incubated for 16 h at 37°C. Cells (1 mL) were then collected by centrifugation at 16,600xg for 1 min and washed with 1 ml of phosphate when buffered saline (PBS). At this point, cell pellets were photographed to compare the staphyloxanthin production. For the extraction of carotenoid pigments, the cell pellets were resuspended in 0.2 mL of methanol by vortexing and this mixture was heated at 55°C for 3 min. Pigment was separated from cell debris by centrifugation at 16,600xg for 10 min. This pigment extraction step was repeated 3 times and the optical densities of collected extractions were measured at 465 nm using a spectrophotometer. Each data point was averaged cells from at least three independent cultures

Antibiotic Susceptibility

Antibiotic Resistance Assay: The standardized Kirby-Bauer disc-diffusion method was performed on Mueller-Hinton agar media using antibiotics Cefoxitin (30mcg), Teicoplanin (30mcg), Linezolid (30mcg) and Penicillin (10 units) and MIC was done for Vancomycin [26,27].

Statistical Analysis

Data were analyzed using SPSS software. Appropriate statistical charts were used to present the data. Chi square analysis was also done. Data was considered statistically significant at the $p < 0.05$ level.

Results

Isolation and Identification of isolates: From a total of 1100 clinical samples, 217 (19.72%) *Staphylococcus aureus* strains were isolated. Among a total of 217 strains of *S.aureus*, 19 (8.7%) were isolated from blood, 07 (3.2%) from body fluids, 93 (42.8%) from pus, 19 (8.7%) from sputum, 02 (1%) from tissue, 02(1%) from tracheal aspirate, 16 (7.3%) from urine and 59 (27.1%) from wound swabs. Among 217 strains obtained, 116 (53.4%) were isolated from females and 101 (46.5%) from males. Out of 217 strains, 143 (65.89) strains were MRSA and rest of 74 (34.10) strains were MSSA.

Among 217 strains, 31 strains (14.29%) were from 0-20 age group, 57 (26.27%) from 21-40 age group, 68 (31.34%) from 41-60, 52 (23.96%) from 61-80 and 9 (4.15%) from age group above 80.

Out of total of 217 strains, 70 were from OPD and rest 147 strains were from IPD. (The data was found to be significant between OPD and IPD patients in case of MRSA and MSSA ($P<0.05$).

Determination of Some Virulence Factors

Coagulase enzyme production :All the isolates had ability to produce free and bound coagulase enzyme.

Hemolysin Production: Different strains of S.aureus showed different types of hemolysis. Out of 217 strains, 42 (19.35%)strains showed only α hemolysin production, 84 (38.70%) strains showed only â hemolysin production and rest 91(41.93%) strains showed both α and β hemolysin production. There was no statistical difference in hemolysin production between MRSA and MSSA isolates ($p > 0.05$) (Table 1).

Biofilm Formation: Among all the strains, 203 (93.54%) were positive for producing biofilm and rest of 13 were negative for producing biofilm and it was found to be statistically significant (p value <0.05). Further it was evaluated that among positive biofilm producers, 62 (28.57%) were weak biofilm producers, 84 (38.70%) were moderate biofilm producers and 57 (26.26%) were strong biofilm producers (Table 2).

Staphyloxanthin Pigment Production: Out of 217 strains of *S.aureus* only 49 (22.58%) strains were able to produce the staphyloxanthin pigment and rest 168 (77.41%) were found to be negative. (Table 3).

Antibiotic Susceptibility assay: All strains of *S.aureus* were sensitive towards Linezolid, Teicoplanin

Table 1: Percentage distribution of hemolysis types among various strains of S.aureus.

	MRSA	MSSA	Total
α hemolysin	26(18.18%)	16(211.62%)	42(19.35%)
β hemolysin	56(39.16%)	28(37.84%)	84(38.70%)
α and β hemolysin	61(42.66%)	30(40.54%)	91(41.93%)
Total	143(65.89%)	74(34.10%)	217(100%)

Table 2: Biofilm produced by *S.aureus* on tissue culture plate.

Biofilm producer	Type	MRSA	MSSA	Total
Negative	No Biofilm	10(6.99%)	03(4.05%)	13(5.99%)
Positive	Weak	46(32.17%)	16(21.62%)	62(28.57%)
	Moderate	45(31.47%)	39(52.80%)	84(38.70%)
	Strong	42(29.37%)	16(21.62%)	57(57.26%)
Total		143(65.89%)	74(34.10%)	217(100%)

Table 3: Percentage of Qualitative detection of staphyloxanthin pigment production of *Staphylococcus aureus* isolates

Staphyloxanthin production	MRSA	MSSA	Total
Positive	33(23.08%)	16(21.62%)	49(22.58%)
Negative	110(76.92%)	58(78.38%)	168(77.41%)
Total	143(65.89%)	74(34.10%)	217(100%)

and vancomycin. On the other hand strains showed high resistance towards Cefoxitin (65.89%). Also, Penicillin was found to be 100% resistant.

Discussion

In this study, the determination of virulence factors like Coagulase activity, hemolysis, biofilm formation, staphyloxanthin production and drug resistance of the *Staphylococcus aureus* clinical isolates was studied [28,29]. In this study it was also found that the prevalence of *S.aureus* infection was more common in case of age group 41-60 yrs (31.34%) and in IPD patients which is usually hospital acquired.

Coagulase enzyme production is used for differentiating the pathogenic *S. aureus* from other strains or species of *staphylococci* In our study all 217 of the isolates were coagulase positive Staphylococcus spp. (CoPS) which represent pathogenic *S. aureus* [19].

In this study, it was that 19.35% *S.aureus* strains had the ability to cause alpha hemolysis on blood agar and 38.70% strains had the ability to cause beta hemolysis and 41.93% had ability to show both alpha and beta hemolysis which shows that most of the strains had capability to produce both alpha and beta hemolysis. There was no statistical difference in hemolysin production between MRSA and MSSA isolates ($p > 0.05$). In a similar study by Franco J.C. et. Al [30]. Hemolysin production was detected in 78% of the *S. aureus* isolates. Fourteen isolates (12%) were alpha hemolysin, thirty-four (29%) beta hemolysin, and forty-four (37%) showed both hemolysins. In another study by V. Pereira et al [31] 81% were demonstrated to be β–hemolytic & 8% were α-hemolytic.

In our study 93.54% of *S.aureus* strains had the ability to produce biofilm, 26.26% of these isolates strong biofilm producers, 38.70% moderate and 28.57% weak biofilm producers Also biofilm production was seen more so in MRSA strains. In a similar study by Khoramian B et al [32] approximately 70% of 215 isolates produced biofilm. Among these, 59.3% were producers of weakly adherent biofilms while 34.8% and 5.8% produced moderate and strong biofilms, respectively.

In this study, 22.58% of *S.aureus* strains had the ability to produce staphyloxanthin. However there was no statistical difference in staphyloxanthin production between MRSA and MSSA isolates ($p > 0.05$). In a study done by Al-Kazaz et. al [33] , 72.1% isolates of *S.aureus* produced the pigment staphyloxanthin.

In our study (65.89%) of *S.aureus* strains were resistant to Cefoxitin. Also Penicillin was found to be 100% resistant. On the other hand Vancomycin, linezolid, Teicoplanin were found 100% sensitive, which indicate that antibiotic vancomycin, linezolid & Teicoplanin could be used as antibiotic treatment for MRSA infection, with a recommendation of investigating an alternative therapeutic agents to avoid the multidrug resistance.

In a study by Emmanuel Onwubiko Nwankwo et al [34] the sensitivity pattern of *S. aureus* to the following antibiotics; Gentamicin, Amoxycillin / clavulanate, Streptomycin, Cloxacillin, Erythromycin, Chloramphenicol, Cotrimoxazole, Tetracycline, Penicillin, Ciprofloxacin, Ofloxacin, Levofloxacin, Ceftriaxone, Amoxycillin and vancomycin were 92.4%, 63.0%, 44.2%, 35.8%, 52.4%, 61.9%, 15.5%, 31.2%, 7.1%, 78.9%, 76.6%, 100%, 71.4%, 30.7% and 100% respectively.

Conclusion

Infection rates due to *Staphylococcus aureus* was higher in elderly age groups and most of the isolates were isolated from IPD suggesting higher rates of infection in hospital setting. Further, higher prevalence of MRSA was seen in IPD settings suggesting an urgent need of infection control practises. Furthermore most of these isolates produced various virulence factors like coagulase, hemolysin production and staphyloxanthin pigment production, which were more common in MRSA than in MSSA. Also, biofilm production was more common in MRSA isolates (65%) suggesting that infection control policies should be adhered positively because biofilm production leads to antibiotic resistance.

References

1. Ryan, KJ; Ray, CG, eds. Sherris Medical Micro-biology (4th ed.). McGraw Hill. 2004. ISBN 0-8385-8529-9.
2. Foster, T.J. The Staphylococcus aureus superbug. J. Clin. Invest. 2004;114:16931696.
3. Lowy, F.D. Staphylococcus aureus infections. N. Engl. J. Med. 1998;339:520-532.
4. Karpatkin, S., and Pearlstein, E. 1981. Role of platelets in tumor cell metastases. Ann. Intern. Med.95:636-641.
5. Nieswandt, B., Hafner, M., Echtenacher, B., and Mannel, D.N. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. Cancer Res. 1999;59:1295-1300.
6. Gorak EJ, Yamada SM, Brown JD. Community-acquired methicillin resistant Staphylococcus aureus in hospitalized adults and children without known risk factors. Clin Infect Dis 1999;29:797-800.
7. Hunt C, Dionne M, Murdock D, et al. Four pediatric deaths from community acquired methicillin-resistant Staphylococcus aureus Minnesota and North Dakota, 1997-1999. JAMA 1999;282:1123-5.
8. Gillet Y, Issartel B, Vanhems P, et al. Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotizing pneumonia in young immunocompetent patients. Lancet 2002;359:753.
9. Mongkolrattanothai K, Boyle S, Kahana MD, Daum RS. Severe *Staphylococcus aureus* infections caused by clonally related community-acquired methicillin susceptible and methicillin-resistant isolates. Clin Infect Dis 2003;37:1050-8.
10. Dufour P, Gillet Y, Bes M, et al. Community-acquired methicillin resistant Staphylococcus aureus infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin Infect Dis 2002;35:819-24.
11. Boussaud V, Parrot a, Mayaud C, et al. Life-threatening hemoptysis in adults with community-acquired pneumonia due to Panton-Valentine leukocidin-secreting *Staphylococcus aureus.* Intensive Care Med 2003;29:1840-3.
12. Foster, T.J. Immune evasion by staphylococci. Nat. Rev. Microbiol. 2005;3:948 958.
13. Manders, S.M. Toxin-mediated streptococcal and staphylococcal disease. J. Am. Acad. Dermatol. 1998;39:383-398.
14. Rooijakkers, S.H., K.P. van Kessel, and J.A. Van Strijp. Staphylococcal innate immune evasion. Trends Microbiol. 2005;13:596-601.
15. Projan, S.J, and R.P. Novick. The molecular basis of pathogenesis, 1997.p.55-81.
16. S. I.Salasia, Z. Khusnan, C.Lammler, M.Zschock. Comparative studies on pheno- and genotypic properties of Staphylococcus aureus isolated from bovine subclinical mastitis in central Java in Indonesia and Hess in Germany, J Vet Sci, 2004;5:103-109.
17. A. Schröder, B. Schröder, B. Roppenser, S. Linder, B. Sinha, R. Fässler, M. Aepfelbacher. Staphylococcus aureus fibronectin binding protein-A induces motile attachment sites and complex actin remodeling in living endothelian cells, MolBiol Cell, 2006;17: 5198-5210.
18. R. M. Corrigan, M. Corrigan, D. Rigby, P. Handley, T. J. Foster. The role of Staphylococcus aureus surface protein SasG adherence and biofilm formation, Microbiology, 2007;153:2435-2446.
19. Brown, D.F.J., D.I. Edwards, P.M. Hawkey, D. Morrison, G.L. Ridgway, K.J. Towner and M.W.D. Wren. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant Staphylococcus aureus (MRSA). J. Antimicrob. Chemother., 2005;56:1000-1018.
20. Quinn, P.J., M.E. Carter,B.K. Markey and G.E. Cartey, Clinical Veterinary Microbiology. Section 2. Bacteriology, 8. Staphylococcus species. Mosby- Year Book Europe Limited, Lynton House, London, England, 1994.p.118-126.
21. Koneman, E.W., S.D. Allen, V.R. Dowell and H.M. Sommer. Diagnostic Microbiology. Chapter 9, 5th ed.,

J.B. Lippincott Co., Philadelphia, USA, 1988. p539-576.

22. Freer, J.H. and J.P. Arbuthnott, Toxins of Staphylococcus aureus. Pharmacol. Ther, 1982;19: 55-106.
23. Pfaller, M.A., D. Davenport, M. Bale, M. Barrett, F. Koontz and R.M. Massanari. Development of. the quantitative micro-test forslime production by coagulase negative staphylococci. Eur. J. Clin. Microbiol. Infect. Dis., 1988;7:30-33.
24. Harborne, J.B. and C.A. Williams. Advances in flavonoid research since 1992. Photochemistry, 2000;55:481-504.
25. Morikawa, K., A. Maruyama, Y. Inose, M. Higashide, H. Hayashi and T. Ohta. Overexpression of. sigma factor, rB, urges Staphylococcus aureus to. thicken the cell wall and to resistb-lactams. Biochem. Biophys Res. Commun., 2001;288:385-389.
26. Clinical and Laboratory Standards Institute (CLSI.). Methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically; approved standard, 8 Ed. CLSI document, Wayne, PA. 2009; M07-A8.
27. Mamishi, S., S. Mahmoudi, R. Sadeghi, et al., 2011. Genotyping of Staphylococcus aureus strains among healthcare workers and patients in the tertiary referral Children's me Th dical Hospital in Tehran, Iran. Br. J. Biomed Sci., 2011;69(4): 173e7.
28. Otto, M., Virulence factors of the coagulase- negative staphylococci.Front. Biosci., 2004;9:841-863.
29. Lee, J.H., J.H. Park,H. Cho and J. Lee. Flavone Reduces the Production of Virulence Factors, Staphyloxanthin and -Hemolysin, in Staphylococcus aureus. Current Microbiology, 2012;65:726-732.
30. Julio C. Franco G. Libertad González V., Sandra C. Gómez M. , Juan M. Carrillo G. and José J. Ramírez. Virulence factors analysis of *Staphylococcus aureus* isolated from bovine mastitis in México., e-Gnosis [online] 2006;6:7
31. Pereira V, Lopes C, Castro A, Silva J, Gibbs P, Teixeira P. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of Staphylococcus aureus isolates from various foods in Portugal. Food Microbiol. 2009 May;26(3):278-82.
32. Khoramian B, Jabalameli F, Niasari-Naslaji A, Taherikalani M, Emaneini M. Comparison of virulence factors and biofilm formation among Staphylococcus aureus strains isolated from human and bovine infections. Microb Pathog. 2015 Nov;88:73-7.
33. Eman J. AL-Kazaz, Alice K. Melconian , Nuha J. Kandela 2014. Extraction of Staphyloxanthin from Staphylococcus aureus Isolated from Clinical Sources to Determine its Antibacterial Activity Against other Bacteria Iraqi Journal of Science, 2014;55(4B):1823-1832.
34. Emmanuel Onwubiko Nwankwo[1] and Magaji Sadiq Nasiru. Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. Pan Afr Med J.; 2011;8:4.

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Microbiological Analysis of Hospital Acquired Infections in Burn Patients

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Abstract

Introduction: About 75% of the mortality associated with burn injuries is related to sepsis, especially in developing countries. Since it is desirable to carry out periodic reviews of bacterial flora of burn wounds and environmental surveillance to look for possible sources of wound contamination, so that preventive strategies could be modified as necessary; present study has been carried out. *Aims & Objectives:* To identify the pathogens causing hospital acquired infections in burn units and determine their antimicrobial resistance pattern. Also to assess & correlate the environmental conditions and risk factors associated with burn infections. *Setting and Design:* Observational study done in rural tertiary care centre for duration of two years. *Materials & Methods:* Patients with Total burn surface area (TBSA)>10% were enrolled in study excluding children less than 12 years of age. From patient; surface wound swab, normal skin swab, nasal swab and throat swab were collected on first, third, seventh, tenth, and fourteenth day post admission. Environmental sampling was done every three months. *Result:* Out of 112 patients included in study, 62 were infected with burn wound infections. Colonization rates were 62% on third day, 77% on 14th day. Rates of invasive infections were 4% on third day increasing to 61% on 14th day. Overall, *Pseudomonas aeruginosa* was the predominant isolate (28%) followed by *Staphylococcus aureus* (23%), *Klebsiella pneumoniae* (11%), Coagulase Negative Staphylococcus (CONS) (9%) & Acinetobacter species (9%). High level of environmental contamination was seen with *Pseudomonas aeruginosa*. *Conclusion:* The most common route of infection was cross-infection.

Keywords: Burn Wound Infections; Colonisation; Environmental Surveillance; Health Care Workers.

Introduction

Burn patients are at a high risk for hospital acquired infection as a result of the nature, the immune-compromising effects of burns, prolonged hospital stays and intensive procedures [1]. Sources of organisms are found in the patient's own endogenous flora, exogenous sources in the environment and healthcare personnel [2]. This observational study was carried out to provide original data on the evolution of microbial flora in burn wounds and its correlation to endogenous & exogenous flora. Environmental conditions & risk factors assessed to know their significance in hospital acquired infections. This will help to undertake appropriate preventive measures to control such infections.

Subjects and Methods

The study conducted at rural tertiary care centre in duration of two years. Oral informed consent obtained from patients or their relatives. All the patients admitted during that period meeting inclusion criteria were included in study. The following inclusion criteria used for the study: 1) Total burn surface area (TBSA) >10% , 2) Length of stay in hospital more than 48 hours, 3) Survival more than 48 hours, 4) Age >12 years and infected as per the criteria of the National Nosocomial Infections Surveillance (NNIS) System [3]. Exclusion criteria: 1) Patients with co-morbidities like Diabetes Mellitus, Chronic Obstructive Pulmonary Disease, AIDS, 2) Patient already having infection on admission. Clinical and demographic details, which included age, sex, burn injury details, all investigations done, procedures and treatment details were filled up in a detailed proforma in consultation with resident doctors. Total surface area burned (TBSA) was calculated by Lund and Browder chart [4]. From patient, surface wound swab, normal skin swab, nasal swab & throat swab collected on first, third, seventh, tenth and fourteenth day post admission and plated on blood agar, MacConkey agar and Sabouraud Dextrose Agar(SDA) agar (Himedia Laboratories, Mumbai). Signs of invasive infections were also noted on day of collection [5]. The samples were collected, processed for aerobic bacterial & fungal identification by standard conventional methods [6,7,8]. Environmental sampling: During the study period, environmental sampling has been done every three months. Settle plates and Swabs were collected from various areas in burn unit, instruments used for dressing, also nasal and skin swabs from Health care workers and caregivers and processed to identify source of infections [7,9]. All the clinical samples and environmental samples tested for antibiotic sensitivity test by Kirby Bauer's disc diffusion method as recommended by the CLSI [2,10]. The procedures followed were in accordance with the ethical standards of the institutional ethics committee. Data generated from this work were tabulated into Microsoft excel sheets and percentage were calculated. No special statistical method used in this study.

Results

This study included 112 cases; total 405 burn wound samples were collected from them on different day of follow ups. In addition, to determine source of isolate, we studied 336 endogenous samples, 36 HCWs samples and 102 environmental samples. 16 patients were discharged before tenth day due to recovery and no infections; so, 96 patients could be followed up to 14th day after admission. Those 16 patients were included as non infected patients.

Table 1 shows patient distribution according to isolation pattern for bacterial isolates from wound samples. 62% of the patients were colonized by the day three, which increased to 77% at the end of second week. 93% (105 cases) patients were colonised on at least one occasion. In 6.25% (7 cases) patients, wounds were sterile on all follow ups. Out of 105 colonised patients, 21 (20%) were multi-microbial on at least one follow up. 4% of the patients were infected on third day increasing to 61% on 14th day. Maximum burn wound infections occurred on seventh day (39 cases). 55% (62 cases) patients suffered from burn wound infection. Out of 62 infected patients, 18(29%) were multi-microbial on at least one follow up.

Table 2 shows colonizers and invading pathogens. *Staphylococcus aureus* is the most common colonizer constituting 34% of all the colonising isolates, while *Pseudomonas aeruginosa* is the most common invader constituting 28% of all isolates. Fungal isolates account for 12% of total isolates. Non albicans Candida spp. found to be the most common fungal isolate in invasive wound infection.

Table 3 shows pathogens isolated from burn wound samples at intervals post-burn. On day three, most of the wounds were colonised by *Staphylococcus aureus,* CONS & *Klebsiella pneumoniae.* Invasive infections occurred in four cases by *Staphylococcus aureus. Staphylococcus aureus* infections increased on day seven & day ten, but got decreased on day 14 due to super-infection of other bacteria or fungi. On day seven; CONS, *Klebsiella pneumoniae & Acinetobacter baumannii* complex started predominating but CONS soon decreased in frequency, while *Klebsiella pneumoniae & Acinetobacter baumannii complex* remained throughout. On day 14, *Pseudomonas aeruginosa* was predominant isolate, followed by *Klebsiella pneumoniae & Acinetobacter baumannii complex.* Sixty four patients were inhabited by same bacteria throughout follow up; while 21 patients showed different isolates on different days. In nine patients, super-infections occurred by other isolates.

Environmental Surveillance

Out of 36 samples collected from healthcare workers, 77.77% were found to be contaminated. Predominant isolate was *Staphylococcus aureus,* followed by CONS. From 78 different environmental

samples that have been processed, 35 (44.87%) were contaminated. Most commonly isolated bacterial species was *Pseudomonas aeruginosa.* Table 4 & 5 summarizes Healthcare workers (HCW) samples & environmental sample culture results, respectively. Table 6 describes source tracing through antibiogram patterns.

Antimicrobial susceptibility results of isolates are graphically presented in Figure 1 to 4. Methicillin Resistant Staphylococcus aureus (MRSA) were predominantly isolated from healthcare workers and environmental samples. Among patients' isolates, rate is 25.49%. Four environmental isolates and four wound isolates were found to be inducible clindamycin resistant. Among all isolates of *Pseudomonas aeruginosa*, 14 wound isolates & four environmental isolates were found to be Multidrug Resistant (MDR). Pan-drug resistant strains were isolated from seven wound samples and five environmental samples from different sites at different periods. Most effective drugs presently are colistin & polymixin B followed by meropenem, imipenem, amikacin, tobramycin. Twenty five percent of *Pseudomonas aeruginosa* isolates were Metallo-beta lactamase (MBL) producers as tested by imipenem-EDTA disk synergy test.

Table 1: Patient distribution according to isolation pattern for bacterial isolates from wound samples

	3rd day	7th day	10th day	14th day	Overall
No. of patients sampled	112	101	96	96	112
No. of patients colonised	69 (62%)	76 (75%)	75 (79%)	74 (77%)	105 (93%)
Polymicrobial wound colonization	5	12	17	1	21
No. of patients with invasive infection	4 (4%)	43 (43%)	59 (61%)	59 (61%)	62 (55%)
Polymicrobial wound infection	0	10	18	12	18
Sterile lesions	43	25	21	22	7

*****Invasive infections include bacterial as well as fungal infections. Colonization include only bacteria, study of fungal colonization is beyond this study.

Table 2: Pathogens isolated from burn wound samples

Pathogens Isolated	Colonization(Co)	Invasive infection(In)
Pseudomonas aeruginosa	40(27.39%)	28(28%)
Staphylococcus aureus	51(34.93%)	23(23%)
CONS	12(8.21%)	9(9%)
Enterococcus spp.	3(2.05%)	0
Citrobacter freundii	7(4.79%)	5(5%)
Acinetobacter baumannii complex	12(8.21%)	9(9%)
Klebsiella pneumoniae	17(11.64%)	11(11%)
Proteus mirabilis	4(2.73%)	3(3%)
Candida albicans	-	2(2%)
Non-albicans Candida spp.	-	5(5%)
Mucor spp.	-	2(2%)
Aspergillus niger	-	2(2%)
Aspergillus flavus	-	1(1%)
Total no. of strains isolated	146	100

Table 3: Pathogens isolated from burn wound samples on different days

Pathogens isolated	3rd day		7th day		10th day		14th day	
	Co	In	Co	In	Co	In	Co	In
Pseudomonas aeruginosa	3	0	14	7	32	26	38	28
Staphylococcus aureus	40	4	34	17	21	17	7	6
CONS	13	0	11	9	4	3	0	0
Enterococcus spp.	3	0	0	0	0	0	0	0
Citrobacter freundii	2	0	3	2	6	4	4	3
Acinetobacter baumannii	1	0	8	6	10	9	12	9
Klebsiella pneumoniae	11	0	16	11	16	11	15	10
Proteus mirabilis	2	0	2	1	4	3	4	3
Candida albicans	-	0	-	0	-	1	-	2
Non-albicans Candida spp.	-	0	-	0	-	3	-	5
Mucor spp.	-	0	-	0	-	0	-	2
Aspergillus niger	-	0	-	0	-	0	-	2
Aspergillus flavus	-	0	-	0	-	0	-	1
Total strains isolated	75	4	88	53	93	77	80	71

Table 4: Culture results of samples from HCWs

Organism	Nail	Hands	Nasal	Total (%)
Pseudomonas aeruginosa	0	1	0	1(3.5%)
Staphylococcus aureus	4	2	6	12(42.85%)
CONS	5	4	2	11(39.28%)
Klebsiella pneumoniae	0	2	2	4(14.28%)
Total strains isolated	9	9	10	28(100%)
Sterile	3	3	2	8
Total samples collected	12	12	12	36

Table 5: Isolates found in environment of burn unit. (6 samples collected from each site during study period)

Sites	Strains Isolated
Settle plate Gen. ward	4 SAU, 1 CONS, 1 KL, 2 ACI
Settle plate Corridor	3 CONS
Settle plate Pvt. Room	4 PAE, 2 SAU, 2 CONS, 1 ACI
Settle plate Dressing	1 PAE, 2 SAU, 1 CONS
Bed	2 PAE, 1 SAU, 1 KL, 3 ACI, 2 CFR
Dressing bed	2 PAE, 1 SAU, 3 KL, 1 ACI
Cheatle forcep soln.	5 PAE, 1 KL
Oxygen mask	2 CONS, 1 KL, 1 ACI
Multidose vial	1 CONS, 1 ACI, 2 CFR
Bronchoscope	3 PAE
Hood	3 SAU
IV stand	3 SAU
Water for bath	2 PAE, 1 ACI, 2 PMI
Total	

SAU: *Staphylococcus aureus,* PAE: *Pseudomonas aeruginosa,* CONS: coagulase negative *Staphylococcus,* ACI: *Acinetobacter baumannii complex,* KL: *Klebsiella pneumoniae,* CFR: *Citrobacter freundii,* PMI: *Proteus mirabilis.*

Table 6: Results of antibiogram typing

Strains		Source
Pseudomonas aeruginosa	17.5%	cheatle forcep, thermometer
	10%	air in pvt room, dressing room, cheatle forceps soln.
	15%	Thermometer
	15%	dressing bed
	17.5%	endogenous flora
	25%	Unknown
*Staphylococcus aureus**	59%	HCW
	14%	Hood, IV stand
	6%	air in ward, dressing bed,
	6%	air in dressing room
	14%	endogenous flora
	8%	Unknown
CONS	100%	Endogenous flora
Citrobacter freundii	50%	patients bed and multidose vial
	50%	Unknown
Acinetobacter spp.	92%	air in general ward, dressing bed, water for bath
	8%	air in pvt room, multidose vial
Klebsiella pneumoniae	29.4%	gen ward dressing bed oxygen mask, Cheatle forcep solution
	23.5%	HCW
	29.4%	endogenous flora
	23.6%	Unknown
Proteus mirabilis	100%	Water for bath

*Total calculation more than 100% due to same strains isolated from more than one source.

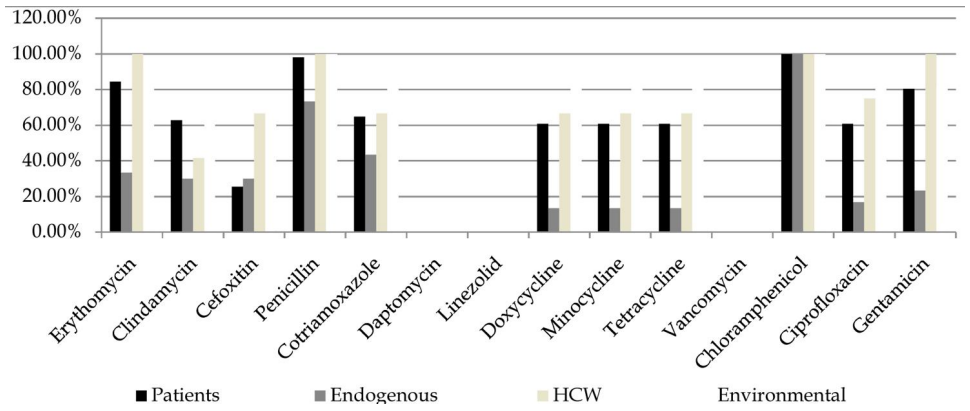

Fig. 1: Antibiotic resistance pattern of *Staphylococcus aureus* isolated from different sources

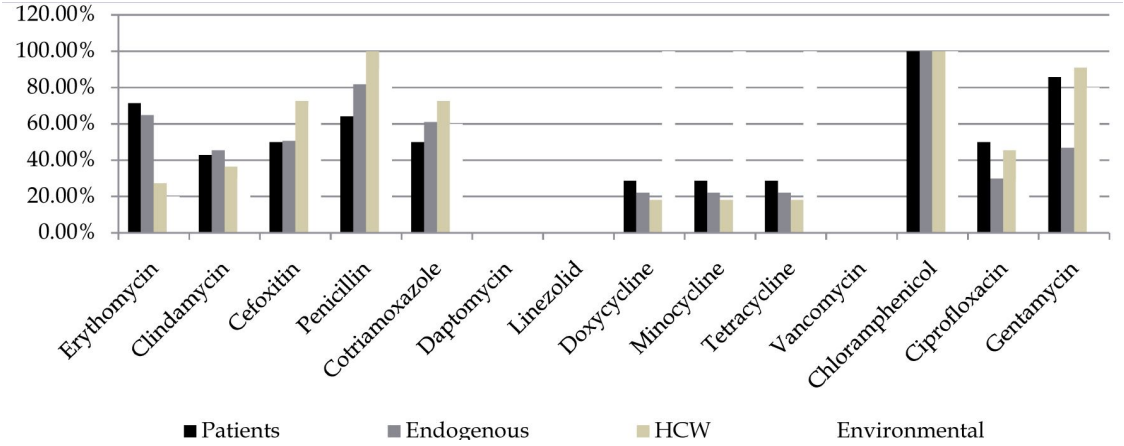

Fig. 2: Antibiotic resistance pattern of Coagulase negative *Staphylococci* isolated from different samples

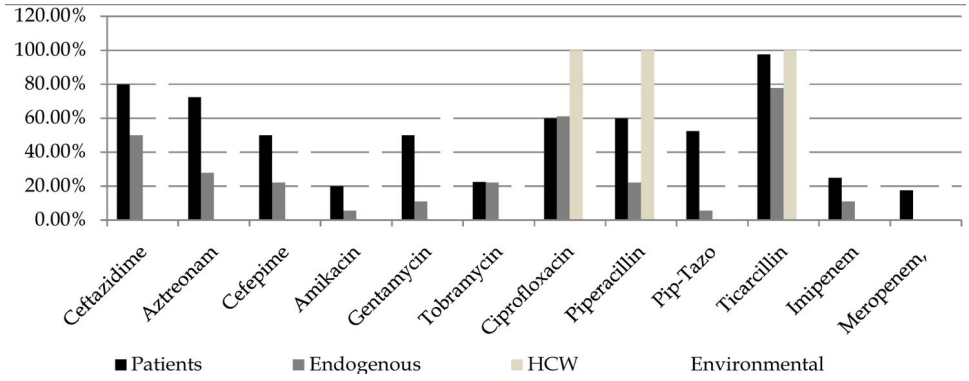

Fig. 3: Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from different sample

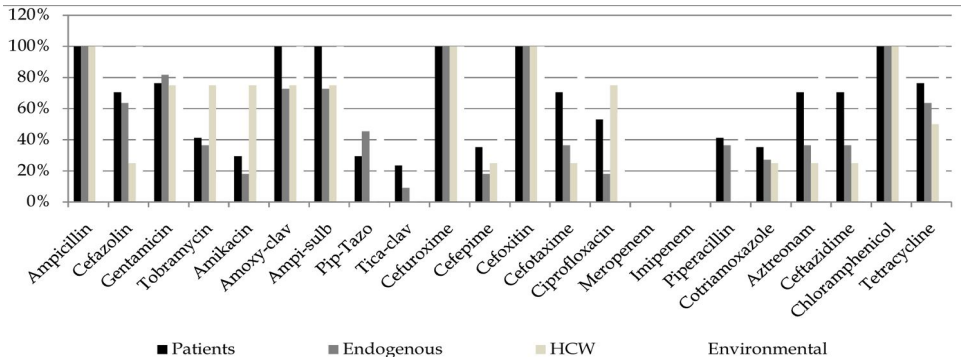

Fig. 4: Antibiotic resistance pattern of *Klebsiella pneumoniae* isolated from different samples

Discussion

The purpose of this study was to analyse the various hospital acquired infections in burn patients, to identify the most common burn pathogens, their sequential emergence in burn wound, antimicrobial resistance of bacteria that causing nosocomial infections and to identify the sources of these pathogens.

Overall, 93% of our study population were colonized at some point of time. After colonization, the organisms on the surface start to penetrate the burn eschar to a variable extent depending on their invasive capacity, local wound factors and the degree of patient's immune-suppression. If viable sub-eschar tissue becomes invaded, disseminated infection is likely to occur [11]. It clearly indicates that if invasion of bacteria has to be decreased; it is very important to maintain the wound sterile from beginning only. Initial colonization gave place for infection. We found that invasive infections started occurring on day seven in most of the cases, a similar finding as found in the study by Taneja *et al* [12].

There was predominance of gram positive bacteria in initial days of admission which got decreased & dominated by gram negative bacteria & fungi in second week.

Staphylococcus aureus (34.93%) was found to be the commonest colonizer followed by *Pseudomonas aeruginosa* (27.39%); finding similar with study by Taneja *et al* [12]. However, predominant bacteria causing invasive infection was found to be *Pseudomonas aeruginosa* (28%). *Pseudomonas aeruginosa* is the most commonly encountered source of chronic or acute burn wound infection in other studies [10,13,14]. The picture is slightly different in China, where *A. baumannii* and *Proteus mirabilis* are the most common causes of burn infection, with *P. aeruginosa* in third place [10,15].

The remarkably high prevalence of *Pseudomonas aeruginosa* in the burn wards may be due to the fact that the organism thrives in a moist environment [16]. *Pseudomonas aeruginosa* is known for its ability to resist killing by a variety of antimicrobials. The minimal nutritional requirements of *Pseudomonas*, as evidenced by its ability to grow in distilled water and its tolerance to a wide variety of physical conditions, contribute to its ecological success and ultimately to its role as an effective opportunistic pathogen.

On second week, fungal species started causing infections. There is a shift from commensal *Candida albicans* to more severe nosocomial pathogens, *non-albicans Candida*. This correlates with the study done by Sarabahi *et al* [17]. Literature showed that fungal colonization frequently associated with the multidrug resistant MRSA and *Pseudomonas* infections demanding treatment with newer generation of antibiotics like imipenem, vancomycin and linezolid [18,19]. In present study, 5 cases showed fungal colonization which were previously infected with various multidrug resistant bacteria. Among them, two were infected with MRSA and three with multidrug resistant *Pseudomonas aeruginosa, Klebsiella spp. and Citrobacter freundii.*

To trace the source of pathogens causing hospital acquired infections; we also studied endogenous flora of skin, nose and throat from patients, resident flora from healthcare workers and environmental samples.

Most of the isolates of *Pseudomonas aeruginosa* were traced to be from environmental source such as water for bath, air sampling, patient bed, cheatle forcep, dressing pad and bronchoscope; whereas *Staphylococcus aureus* was mainly through the healthcare workers. Coagulase negative *Staphylococcus* spp. from wound infections were found to be from patients' own endogenous flora. *Citrobacter freundii, Acinetobacter* spp. and *Proteus mirabilis* were traced to be from various environmental sources. For *Klebsiella pneumoniae,* all sources were found to be of equal importance (Table 6). Taneja *et al* found high contamination of air and surfaces with *Staphylococcus aureus.* They found no environmental sources for *Pseudomonas aeruginosa* which is contradictory to our findings [12].

In our study, we found most of the environmental samples and HCW samples contaminated. Hence, periodic surveillance and standard precautions to prevent cross infection should be carried out routinely. Hand hygiene for healthcare workers is essential to prevent cross infections. Their periodic trainings and periodic surveillance of their flora should be done. As in our study, surfaces and air is mostly contaminated, so thorough cleaning and fumigation is a must. Barrier precautions must be taken. Infected patients should be cared in separate wards by the separate healthcare workers. Dressing of uninfected patients should be done before infected patients. Regular disinfection of bed linens should be done to prevent cross transmission to other patients.

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References

1. Oncul O, Acar EUA, Turhan V, Yeniz E, Karacaer Z, Yildiz F. Prospective analysis of nosocomial infections in a Burn Care Unit , Turkey. indian j med res. 2009 Dec;130:758–64.
2. Weber J, McManus A. Infection control in burn patients. Burns. 2004.p.16–24.
3. Sudarmono P, Wiwing V. Hospital Acquired Bacterial Infection in Burns Unit at Cipto Mangunkusumo Hospital , Jakarta. Microbiol Indones. 2007;1(1):23–6.
4. Lund CC. The estimation of areas of burns. Surg Gynecol Obste. 1944;79:352-8.
5. CDC/NHSN. CDC / NHSN Surveillance Definitions for Specific Types of Infections [Internet]. 2014. p. 17–1 to 17–63. Available from: www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf.
6. National Health and Nutrition Examination Survey Specimen Collection Procedures. 2000.p.2-3.
7. Pasquarella C, Pitzurra O, Savino A. The index of microbial air contamination. J Hosp Infect. 2000 Dec;46(4):241-56.
8. Winn WC, Allen SD, Janda WM, Koneman EW, Procop G, Schreckenberger PC, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams & Wilkins; 2006.p.67-110.
9. Taneja N, Chari PS, Singh M, Singh G, Biswal M, Sharma M. Evolution of bacterial flora in burn wounds/ : key role of environmental disinfection in control of infection. 2013;3(2):102–7.
10. Branski LK, Al-Mousawi A, Rivero H, Jeschke MG, Sanford AP, Herndon DN. Emerging infections in burns. Surg Infect (Larchmt). 2009;10(5):389–97.
11. Soares De Macedo JL, Santos JB. Bacterial and fungal colonization of burn wounds. Mem Inst Oswaldo Cruz. 2005;100(5):535–9.
12. Taneja N, Chari PS, Singh M, Singh G, Biswal M, Sharma M. Evolution of bacterial flora in burn wounds: key role of environmental disinfection in control of infection. Int J Burns Trauma [Internet]. 2013;3(2):102–7. Available from: http://www.pubmedcentral.nih.gov/articlerender. fcgi?artid= 3636666& tool=pmcentrez& rendertype= abstract.
13. Weinstein R a, Mayhall CG. The Epidemiology of Burn Wound Infections: Then and Now. Clin Infect Dis [Internet]. 2003;37(4):543–50. Available from: http://cid.oxfordjournals.org/content/37/4/543.abstract.
14. Pruitt B a, McManus a T, Kim SH, Goodwin CW. Burn wound infections: current status. World J Surg. 1998;22(2):135–45.
15. Wang Z, Rong X, Zhang T, Liu L. [Distribution and drug resistance analysis of bacteria in different wound infections]. Nan Fang Yi Ke Da Xue Xue Bao [Internet]. 2009;29(1):82–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19218119.
16. Song W, Lee KM, Kang HJ, Shin DH, Kim DK. Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. Burns [Internet]. 2001 Mar;27(2):136–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0305417900000863.
17. Sarabahi S, Tiwari VK, Arora S, Capoor MR, Pandey A. Changing pattern of fungal infection in burn patients. Burns. 2012;38(4):520–8.
18. Goyal NK, Gore M a., Goyal RS. Fungal colonisation in burn wounds: An Indian scenerio. Indian J Surg. 2010;72(1):53–6.
19. Cochran A, Morris SE, Edelman LS, Saffle JR. Systemic Candida infection in burn patients: a case-control study of management patterns and outcomes. Surg Infect [Internet]. 2002;3(4):367–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12697083.

Original Article

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Unraveling the Molecular Diversity of Lipid Degrading Enzymes from Microbial Consortia of a Cold Desert from India

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Abstract

Metagenomics has emerged as one of the important tools that can endow bottomless insights about the molecular diversity, distribution, ecological roles, microbial grouping and fastidious biological functions of microorganisms in a particular environment. In the present investigation, we explored metagenomics to delineate the molecular diversity of lipid degrading enzymes from one of the cold deserts located at Drass region of Jammu, India. Lipases have been implicated in number of industrial applications that mainly include the food, detergents and bioremediation of the oil contaminated sites. To meet our aim, we used degenerate primers to amplify conserve domain of lipase that encode the catalytic site, from the environmental DNA, followed by pyrosequencing and analysis of the amplified DNA fragments. Interestingly, analysis of the sequencing report revealed diverse classes of lipases from variety of microorganisms, many of which were observed to be unique, having no known representative in the gene database, and henece considered to be novel. The sequenced fragments were aligned and translated into the amino acid sequences, the average length of the translated amino acid sequences were observed to be ~60-100 amino acids that demonstrated~ 40-99% homology with the existing lipases. Phylogeny tree constructed revealed origin and clustering of these lipases. Principle component analysis further confirms the clustering and variations among these sequences, statistically.

Keywords: Metagenomics; Lipase; Cod Desert; Microbial Community.

Introduction

Industrial enzymes constitute largest segment with revenues of nearly $1.2 billion in 2011 that was expected to grow ~$1.8 billion by 2016 [1]. Hydrolytic enzymes, such as lipases, pectinases, amylases, oxidases and esterases occupied major share in industrial enzyme market. Rapid increase in environment awareness at government and regulatory levels, several industries are employing biocatalysts for their promising applications. The chemical industries, in particular are using biocatalysts for their promising applications in molecular, region and enantio-selectivity. The organic reactions performed by the biocatalysts are vast, and is the first rate-limiting step in the iterative cycle of setting up a bio-catalytic process. Therefore, identification of appropriate enzymes, capable of catalyzing reaction under desired environmental conditions is of prime importance [2]. Lipid digesting enzymes comprises a group of enzymes that catalyzes the hydrolysis as well as synthesis of triacylglycerols, under aqueous and non-aqueous system respectively. At structural

level, all these lipases shared similarity in terms of their catalytic triad that primarily consists of the amino acids serine, histidine and aspartate (or glutamate). The serine residue is located strictly in the conserved β-ε-Ser-α motif. These enzymes are distributed widely throughout the earth's flora and fauna. More abundantly, however, they are found in bacteria, fungi and yeast [3]. Molecular investigation of various ecosystems employing 16S rRNA studies have shown that one gram soil may contain up to ten billion microorganisms of possibly thousands of different species [4]. Interestingly, only small fraction of diversity present in most habitats could be cultivated for study in pure culture [5, 6] and thus results in loss of major portion of the microbial diversity expected to harbor number of genes with abundant bio-catalytic potential [7]. These uncultivable communities can be prospected *via* culture independent approach [8]. Drass, a cold desert present an excellent example of the extreme environment, the microbes here must have adopted mechanism to subsist under extreme cold conditions, for performing various functions. It offers opportunities to explore the associated molecular diversity of cold adapted biocatalysts and their origin that can be used in various industrial applications. Previous studies have also documented that the psychrophilic microorganisms as well as enzymes derived from them can be used as an alternative to physicochemical methods for the bioremediation of solids and waste waters polluted hydrocarbons [9]. Here, we embarked upon demarcating the molecular diversity of different lipid digesting enzymes *via* gene targeted metagenomics that provided deep insights about their diversity, distribution and the ecological roles they are playing in such extreme environment conditions. We further concentrate our discussion towards finding relationships between microbial assemblages by constructing a phylogeny tree followed by PCA analysis of the diverse sequences. This study can provide a podium to screen novel cold lipid degrading enzymes from cold environment that in future could be employed in various industrial settings.

Experimental Procedures

Collection of Soil Sample and Metagenomic DNA Extraction

The soil samples were collected from different location of Drass Mountains, located in North Himalayan range (J&K, Ladakh) (34.45^{0}N, 75.77^{0}E) during May 2010, and were amalgamated. The samples were collected aseptically in plastic bags by digging soil surface to 1 cm depth. Hands, trowels were treated with 70% ethanol before use. The samples were transported to the laboratory in ice and stored at -20^{0}C. To recover maximum microbial diversity of soil samples, the metagenomic DNA was extracted by employing four methods reported previously [10-13]. The extracted DNA was checked for the quantity and the quality respectively, before PCR amplification.

PCR Amplification and Pyrosequencing

The extracted DNA (1:500) from composite soil was used as template for the PCR amplification; a touchdown PCR was performed to amplify the conserve domain of the lipases. The thermal cycling for the touchdown PCR was adjusted as described previously [14]. A highly degenerate set of primers LipF (5'-GACCRATYGTSCTSGTVCAYGG-3') and LipR2 (5'-GCCRCCSTGRCTRTGRCC–3') was used to amplify the diverse class of the lipases gene fragments. These primers were designed by aligning the amino acid sequences of true lipases encoded by family 1 and subfamilies 1-1V, and the most conserved regions showing highest level of conservation were identified as the nucleic acid spanning the oxyanion hole and the active site of the lipolytic enzymes as evidenced from the thesis published by Smith et al. 2006 [15]. PCR Amplified products were visualized on an agarose gel, and DNA band corresponding to 200 bp and 500bp were excised, pooled and purified using TaKaRa Agarose Gel DNA Purification kit. All amplified DNA fragments were pooled and pyrosequenced in research and testing lab (company providing commercial services).

Sequence Analysis and Construction of Phylogenetic Tree

The sequencing report was analyzed and the raw sequences were processed and analyzed. The sequences were trimmed by removing the primers sequences, and a minimum length of 200bp was considered for analysis purpose. The repetitive sequences were filtered by OTU filtering, employing BLASTCLUST. Barcode Sequence and linker Primer sequence were trimmed from raw data. The evolutionary history was inferred using the Neighbor-Joining method [16]. The optimal tree with the sum of branch length=2.24473846 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method [17,18] and are in the units of the number of base substitutions per site. The analysis involved 467 nucleotide sequences. Codon positions included were 1st+2nd+3rd+ Noncoding. All positions containing

gaps and missing data were eliminated. There were a total of 1 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [18].

PCA Analysis

Additionally, a bilinear multivariate modeling (AIBIMM) alignment independent method was used to analyze microbial community encoding tri acylglycerol hydrolases, as described previously [19]. All lipase sequences were transformed into multimer frequencies ($n = 6$) by computer program PhyloMode (http:// www. matforsk. no/ web/ sampro. nsf/ downloadE/ Microbialcommuni ty). Next, the multimer frequency data was compressed using principal component analysis (PCA). The starting and end point in the sequences were corrected using the normalize option data in PhyloMode. The two first principal components (PCs) were subsequently used for generating pair wise Euclidean distances for phylogenetic tree construction.

Nucleotide Sequence Accession Numbers and Protein Homology Search

The lipid degrading nucleotide sequences analyzed here have been retrieved from the NCBI SRA file having accession number of SRP047100 and the BioProject PRJNA260660. Next to get insights into the % homology and the conserve folds in their structure, the individual amino acid sequences were blasted manually at the blastP site, followed by analysis of these lipases for the presence of type of conserve fold.

Results and Discussion

Understanding molecular diversity *via* conventional screening involves culturing individual microbes in suitable medium, followed by extraction and manipulation of the DNA. Unfortunately, such screening method does not allow complete accessibility to the microbial diversity of that particular environment, and thus their genomic pool largely remain uncharted, as reported previously in several studies. This anomaly is primarily attributed to lack of culturing 90-95% microorganism from any environment. High-throughput targeted metagenomics of the desired amplified genes, followed by next generation sequencing (NGS) can provide deep insights about the molecular diversity. This study was aimed to unravel the molecular diversity of cold adapted lipid degrading enzymes that may serve the basis of screening novel cold adapted biocatalysts. These cold adapted lipases have found immense applications in various industrial settings ranging from food, detergents and in the bioremediation processes [20,21]. This is the first report of amplicon pyrosequencing of lipolytic genes from soil metagenome of Drass, a cold desert. To uncover maximum diversity from Drass, the soil samples were collected from various locations of Drass regions followed by extraction of DNA by four different methods that resulted in extraction of high molecular weight metagenomic DNA (data not shown). Touchdown PCR amplification of the metagenomic DNA with highly degenerate primer (Designed to target the conserve region of the lipases as mentioned in materials and methods) showed DNA amplification corresponding to 200 and 500 bp. The degenerate primer set used in the present study were previously used to explore lipases diversity of Antarctic mineral soils [15]. Additionally, several other studies also reported successful use of degenerate primers to target specific genes within the environmental DNA [22-25]. Degenerate primers potentially allows access to target genes within the community, though the efficacy is dependent on the level of degeneracy of the primers and the known sequences used as template during primer design [26]. Interestingly, in addition to the lipases, other sequences having α/β hydrolases were also found, as they harbor amino acids sequences similar to the lipases [27]. However, they were excluded from discussion. Sequence analysis showed that majority of the lipase producers were of *Proteobacterium* origin (~48%), followed by *Actinobacterium* (21%), *Firmicutes* (12%), *Bacteroids* (3.75%), *Cyanobacteria* (1.04%), *Planctomycetes* (1.04%) *Paenibacillaceae* (0.83%), *Chloroflexi* (0.21%), *Spirochaetes* (0.21%), *Atribacteria* (0.21%). And more interestingly, the uncultured bacterial population having no known representative in the gene databases were also observed (7.5%) (Figure 1A). Furthermore, at the genus level, total of 73 genera producing lipases were identified. Interestingly, various *Pseudomonas* species were identified to be the major producer of the lipid degrading enzyme in this cold environment that contributed ~15% of the total producer, followed by *Desulfosporosinus* and uncultured bacterial species that contributed ~8%, the *Oxalobacteraceae*bacterium represented 7% and so on. *Sphingomonas* represented nearly 2% of the lipase producers, *Vibrio* represents nearly 5% of the total producer and so on, the detail about the abundance at the species level is presented in the Figure 1B and supplementary Table S1. A phylogeny tree constructed based on the maximum parsimony methods demonstrate the level of similarities these sequences had shared. The tree was

displayed as radial tree (Figure 2). Furthermore, the PCA analysis was done, where the primary component analysis demonstrated the clustering of various lipase sequences. This multivariate statistical approach was used to explore the distribution patterns of bacteria. The primary objective of doing PCA analysis was to gather major information of the data and expressing it as a set of new orthogonal variables (principal components). PCA gives more comprehensive interpretation of the relationship among microorganisms. Principle component analysis (PCA) of recovered sequences by non-alignment fashion showed uniqueness of the recovered clones. The PC1 and PC2 showed 11.94 and 4.12% variance (Figure 3). The lipase sequences analyzed in the present investigation on an average had a length of 60-100 amino acids long. Among various lipases identified, lactonizing lipases, lysophopolipases, tricylglycerol hydrolases, putative lipases and their precursors were observed to be present most abundantly in this desert. BLASTp analysis carried out against lipase engineering database (LED) with a cut-off e-value of 1e-5 [28] revealed that the identified lipase from Drass metagenome assigned two classes having GX and GGGX motifs in their oxyanion holes [29], a conserve domain known to stabilize the negatively charged transition state during enzymatic hydrolysis of the

Table 1:

Serial No.	Name of Bacteria	Abundance
1.	Acidovorax sp.	17
2.	Aeromonas sp.	3
3.	Alcanivorax	14
4.	AliiVibrio wodanis	1
5.	Amycolatopsis azurea	3
6.	Arenimonas oryziterrae	30
7.	Arthrobacter arilaitensis	1
8.	Atribacteria bacterium	1
9.	Bacillus sp.	5
10.	Burkholderia sp.	4
11.	Caldimonas manganoxidans	3
12.	Candidatus Accumulibacter sp. SK-11	5
13.	Chondromyces apiculatus	1
14.	Chromobacterium violaceum	1
15.	Clostridium sp.	5
16.	Cohnella panacarvi	13
17.	Collimonas Fungivorans	2
18.	Corallococcus coralloide	2
19.	Corynebacterium aurimucosum	1
20.	Curtobacterium ginsengisoli	1
21.	Dechloromonas aromatica	4
22.	Desulfosporosinus	39
23.	Fulvivirga imtechensis	14
24.	Geodermatophilaceae bacterium	1
25.	Glaciecola sp.	2
26.	Gordonia aichiensis	1
27.	Gryllotalpicola ginsengisoli	2
28.	Hahella **chejuensis**	2
29.	Herbaspirillum seropedicae	1
30.	Janibacter sp.	1
31.	Janthinobacterium lividum	9
32.	Leptolyngbya sp.	2
33.	Leptospira inadai	1
34.	Mariniradius saccharolyticus	4
35.	Marinomonas sp.	1
36.	Massilia sp.	1
37.	Mesorhizobium sp.	1
38.	Microbacterium laevaniformans	1
39.	Moritella sp.	2
40.	Myxococcus sp	2
41.	Nitrosospira multiformis	1
42.	Nocardia sp.	2
43.	Nocardiopsis sp.	2
44.	Oleispira Antarctica	1
45.	Oxalobacteraceae bacterium	34

46.	Oxalobacteraceae bacterium AB_14	3
47.	PaeniBacillus dendritiformis	11
48	Patulibacter	7
49.	Photobacterium sp.	5
50.	Plesiocystis pacifica	2
51.	Pseudoduganella violaceinigra	4
52.	Pseudogulbenkiania Ferrooxidans	1
53.	Pseudomonas sp.	74
54.	Psychrobacter sp.	4
55.	Ralstonia sp.	5
56.	Ramlibacter tataouinensis	1
57.	Rhodococcus sp.	8
58.	Rhodoferax ferrireducens	4
59.	Rhodopirellula sallentina	1
60.	Rubrivivax gelatinosus	6
61.	Rubrobacter xylanophilus	5
62.	Saccharomonospora marina	1
63.	Salinispora arenicola	1
64.	Singulisphaera acidiphila	4
65.	Sorangium cellulosum	1
66.	Sphingomonas sp.	10
67.	Sphingopyxis sp	1
68.	Spirosoma linguale	1
69.	Streptomyces albulus	1
70.	Streptomyces albulus	2
71.	Thalassolituus sp.	9
72.	Uncultured	39
73.	Vibrio sp.	25

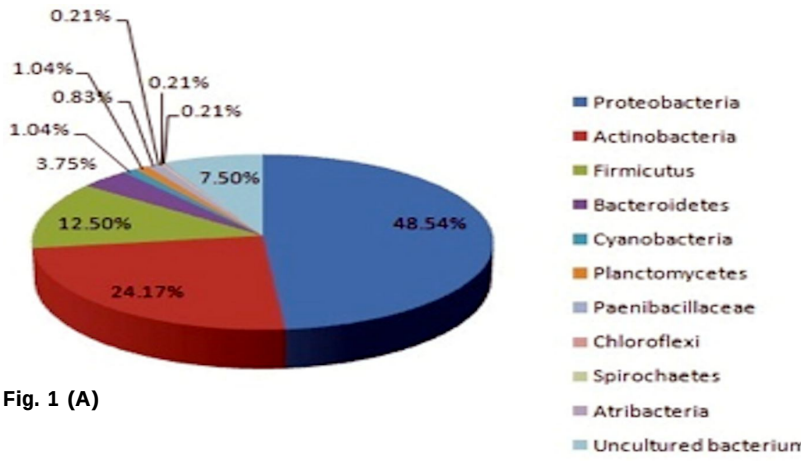

Fig. 1 (A)

Fig. 1 (A): % abundance of the phylum producing lipid degrading enzymes from Drass region

Fig. 1(B): % abundance of lipase producer at the species level.

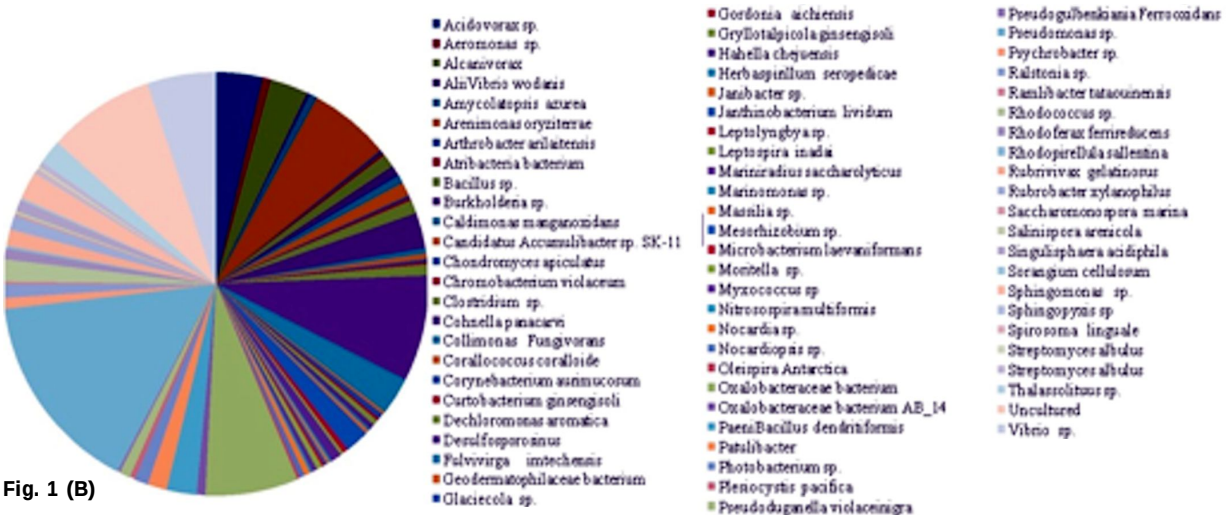

Fig. 1 (B)

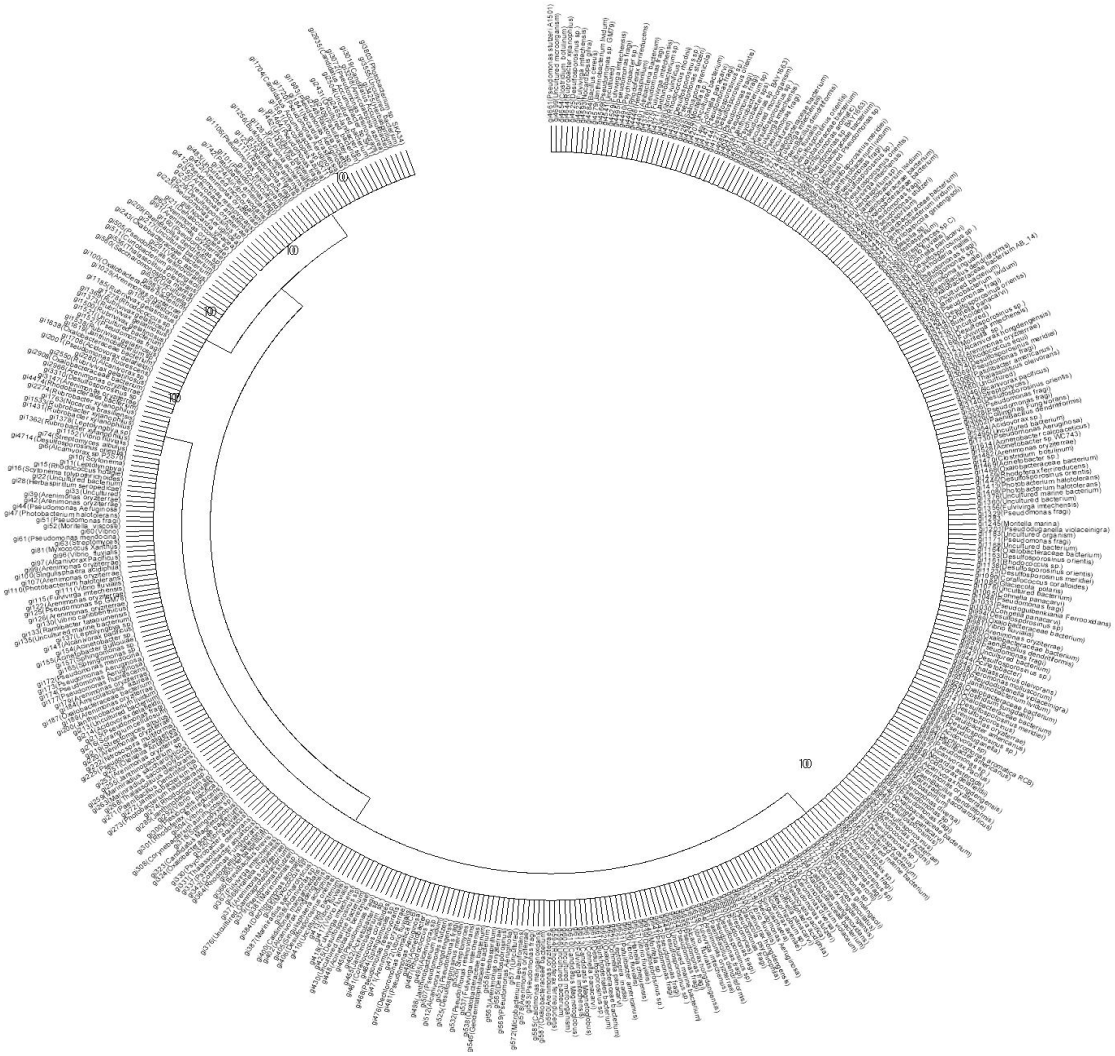

Fig. 2: Phylogenetic tree of the annotated sequences of the lipid degrading enzymes analyzed after PCR amplification of the DNA with the degenerate primer followed by pyrro-sequencing, evolutionary history was inferred by maximum parsimony method from MEGA6 programs (Tamura et al. 2013) using 1,000 bootstrap replicates.

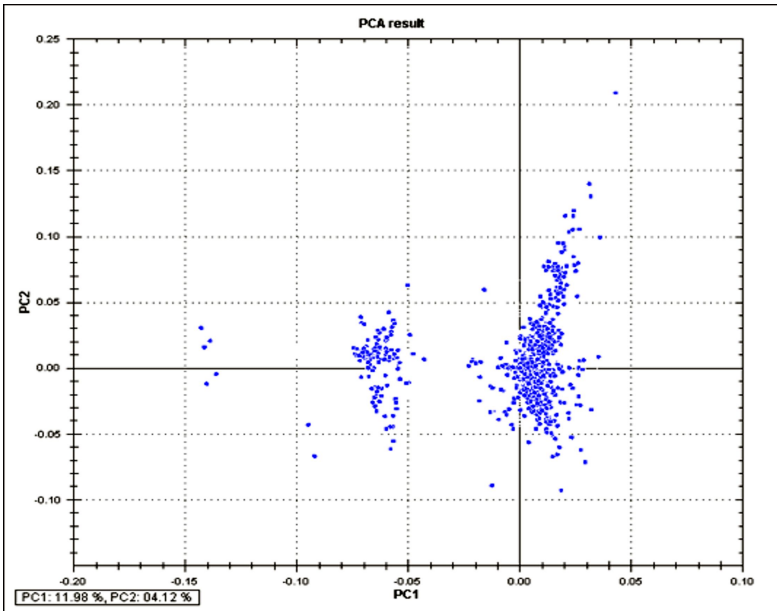

Fig. 3: PCA analysis for lipid degrading enzymes. The first principal component (PC1) is the line explaining 11.94 % variance. The second principal component (PC2) is the line that explains 4.12 % variance

substrate [30]. Of the particular note, the lipase sequences analyzed showed ~40-99% homology with the earlier reported lipases, and were observed to be closely related with the lipase esterase super family. Carboxylic esters (EC: 3.1.1.1), and their catalytic apparatus involves three residues (catalytic triad): a serine, a glutamate or aspartate and a histidine. These catalytic residues are responsible for the nucleophilic attack on the carbonyl carbon atom of the ester bond. In contrast with other alpha/beta hydrolase fold family members, p-nitrobenzyl esterase and acetylcholine esterase have a Glu instead of Asp at the active site carboxylate. Further analysis of lipases for their respective classes revealed that they can be annotated as *Burkholderia* lipases (abH15) (~61.58%), *Streptomyces* lipases (abH16) (~18.75%), *Bacillus lipases* (abH18) (~8.7), *Candida antarctica* lipases (abH37) (~4.59%), *Moraxella* lipases (abH07) (~2.65%), *Cytosolic hydrolases* (abH08) (~2%), *Moraxella* lipase 2 like (abH04) (~0.36%). The superfamily abH15 was predominantly distinguished into two homologous families abH15.01 (1.84%) and abH15.02 (59.74%) affiliated to *Staphylococcus aureus* lipase and *Burkholderiacepacia* lipase. Cytosolic hydrolases (abH08) with homologous family abH08.13 (soluble esterases / lipases / peptidases) were included as these sequences have shared approximately 25% of amino acid identity with lipases belonging to the family V [31,32]. Further, it was observed that the adaptation of various phylum in cold environment may be attributed to the production of Exopolysaccharides (EPS) [33] and various other chaperone proteins implicated in adaptation to cold stress conditions [34,35]. Interestingly, several genes from the cold adapted environments have been elucidated in the major bacterial phyla of *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Actinobacteria*, *Cyanobacteria*, etc, and the genes encoding cold stress proteins are of particular note in *Proteobacteria* and *Cyanobacteria* as previous studies have shown that the two bacterial phyla had a higher percentage of these genes in ice cold polar regions [36].

In addition, the present study also demonstrated the presence of phylum *Atribacteria* in the cold region that primarily has been identified from hot spring environments, sediments and geothermal systems [37] as well as from Antarctica, with a high abundance in the methane rich zones [38]. *Pseudomonas* is the predominant species found in the soil of Drass that is known widely to produce cold adapted lipases [39], and have been reported in literature as one of the active oil degraders in cold environment [40,41]. The present metagenome data was also compared with other studies being carried out based on the cultivation dependent approaches from Drass, and it was observed that the *Acinetobacter calcoaceticus, Arthrobacter agilis, Pseudomonas sturtzi, Pseudomonas psychrotolerans, Rhodococcus erythropolis* were the common lipase strains [42]. More interestingly, when compared with previous culture–independent studies [43-45], far more lipase genes diversity could be observed in the present study, which is phylogenetically more diverse suggesting PCR-based approach as an important and efficient way to identify lipase genes in extreme environments. The partial lipase gene fragments obtained can further be used as probe to screen the possible full-length genes from a metagenomic library constructed from soil of any extreme cold condition. Further, it is suggested that to improve the more diversity of the lipid degrading enzymes different primer sets should be used with similar function from distant families or genes resulting from convergent evolution are unlikely to be detected using a single gene-family-specific set of primers. Moreover Gene specific PCR only yields a partial gene fragments, requiring additional steps to obtain the up- and down-stream flanking regions. In such instances, the partial gene fragment can be used as a probe to identify the full length gene.

Conclusion

The present study report molecular diversity of lipid degrading enzymes from metagenome source of Drass, a cold desert. Such study may provide basis of comprehensive screening of novel cold adapted lipid degrading enzymes from metagenomic library constructed from cold environment.

References

1. Adrio JL, Demain AL .Microbial Enzymes: Tools for Biotechnological Processes. Biomolecules 2014;4: 117-139.
2. Rasmussen LE, Xu C, Sørensen JF, Nielsen MK and Meyer AS. Enzyme kinetics and identification of the rate-limiting step of enzymatic arabinoxylan degradation. Biochem Eng J 2012;69:8–16.
3. Jaeger KE, Ransac S, Dijkstra BW, Colson C, Van Heuvel M, Misset, O. Bacterial lipases. FEMS Microbiol Rev 1994;15:29–63.
4. Knietch A, Waschkowitz T, Bowien S, Henne A, Daniel R. Metagenomes of complex microbial consortia derived from different soils as sources for novel genes conferring formation of carbonyls from short-chain polyols on Escherichia coli. J Microbiol Biotechnol. 2003;5:46-56.

5. Kaeberlein T, Lewis K, Epstein SS. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. Science. 2002;296: 1127-9.
6. Stewart EJ. Growing Unculturable Bacteria. J Bacteriol. 2012;194: 4151-4160.
7. Lu S, Park M, Ro HS, Lee DS, Park W, Jeon CO . Analysis of microbial communities using culture-dependent and culture-independent approaches in an anaerobic/aerobic SBR reactor. J Microbiol. 2006;44: 155-61.
8. Gupta P, Vakhlu J. Bacterial diversity of Drass, cold desert in Western Himalaya and its comparison with Antarctic and Arctic. Arch Microbiol.2015;197:851-60.
9. Margesin R, Miteva V. Diversity and ecology of psychrophilic microorganisms. Res Microbiol. 2011; 162:346-61.
10. Zhou J, Bruns MA, Tiedje JM. DNA recovery from soils of diverse composition. Appl Environ Microbiol. 1996;62:316-322.
11. Wechter P, Williamson J, Robertson A, Kluepfel D. A rapid, cost-effective procedure for the extraction of microbial DNA from soil. World J Microbiol Biotechnol. 2003;19:85-91.
12. Sharma PK, Capalash N, Kaur J. An improved method for single step purification of metagenomic DNA. Mol Biotechnol. 2007;36:61–63.
13. Pang M, Abdullah N, Lee C, Ng CC. Isolation of high molecular weight DNA from forest topsoil for metagenomic analysis. Asia Pac J Mol Biol Biotechnol. 2008;16:35-41.
14. Kaur G, Sharma R, Singh K, Sharma PK. Delineating bacterial community structure of polluted soil samples collected from cancer prone belt of Punjab, India. 3 Biotech. 2015;5:727-734.
15. Smith J. Microbial diversity and gene mining in Antarctic soils. Ph.D. thesis. University of the Western Cape, Cape Town, South Africa. 2006.
16. Saitou N, Nei M. The neighbour-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406-425.
17. Tamura K, NeiM, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci (USA) 2004;101: 11030-11035.
18. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 2013;30:2725-2729.
19. Rudi K, Zimonja M, Næs T. Alignment-independent bilinear multivariate modelling (AIBIMM) for global analyses of 16S rRNA gene phylogeny. Int J Syst Evol Microbiol. 200;56:1565–1575.
20. Hasan F, Shah AA, Hameed A. Industrial applications of microbial lipases. Enzyme Microb Technol. 2006; 39:235–251.
21. Margesin R, Feller G. Biotechnological applications of psychrophiles. Environ Technol. 2010;31:835–844.
22. Kotik M, Stepanek V, Maresova H, Kyslik P, Archelas A. Environmental DNA as a source of novel epoxide hydrolase reacting with aliphatic terminal epoxides. J Mol Catal B: Enzym 2009;56:288–29.
23. Wang G, Luo H, Meng K, Wang Y, Huang H, Shi P, Pan X, Yang P, Diao Q, Zhang H, Yao B . High Genetic Diversity and Different Distributions of Glycosyl Hydrolase Family 10 and 11 Xylanases in the Goat Rumen. PLoS ONE. 2011;6:e16731.
24. Wang G, Huang X, Ng TB, Lin J, Ye XY . High Phylogenetic Diversity of Glycosyl Hydrolase Family 10 and 11 Xylanases in the Sediment of Lake Dabusu in China. PLoS ONE 2014;9:e112798.
25. Parra LP, Espinaa G, Deviaa J, Salazara O, Andrewsa B, Asenjoa JA. Identification of lipase encoding genes from Antarctic seawater bacteria using degenerate primers: Expression of a cold-active lipase with high specific activity. Enzyme Microb Tech. 2015;68:56–61
26. Cowan D, Meyer Q, Stafford W, Muyanga S, Cameron R, Wittwer P. Metagenomic gene discovery: past, present and future. Trends Biotechnol. 2005;23:321-329.
27. Bell PJL, Anwar S, Moreland DG, Natalie CC, Helena N, Peter LB. Prospecting for novel lipase genes using PCR. Microbiology 2002;148:2283–2291.
28. Mendes LW, Kuramae EE, Navarrete AA, Van V, Tsai. Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 2014;8: 1577–1587.
29. Pleiss J, Fischer M, Peiker M, Thiele C, Schmid RD. Lipase engineering database-Understanding and exploiting sequence-structure-function relationships. J Mol Catal B Enzym 2000;10:491-508.
30. Anobom CD, Pinheiro AS, De-Andrade RA, Aguieiras ECG, Andrade GC, Moura MV, Rodrigo VA, Freire DM. From Structure to Catalysis: Recent Developments in the Biotechnological Applications of Lipases. BioMed Res Int 2014; ID. 684506:1-11.
31. Arpigny JL, Jaeger KE . Bacterial lipolytic enzymes: classification and properties. Biochem J. 1999;343:177-183.
32. Tirawongsaroj P, Sriprang R, Harnpicharnchai P, Thongarama T, Champreda V, Tanapongpipatn S, Pootanakit K, Eurwilaichitr L. Novel thermophilic and thermostable lipolytic enzymes from a Thailand hot spring metagenomic library J Biotechnol 2008; 133:42–49.
33. Poli A, Anzelmo G, Nicolaus B. Bacterial Exopolysaccharides from Extreme Marine Habitats: Production, Characterization and Biological Activities. Mar Drugs. 2010;8:1779-1802.
34. Goldstein J, Pollitt NS, Inouye M. Major cold shock protein of Escherichia coli. Proc Natl Acad Sci USA. 1990;87:283–287.
35. Phadtare S, Inouye M. Genome-wide transcriptional

analysis ofthe cold shock response in wild-type and cold-sensitive, quadruple-csp-deletion strains of Escherichia coli. J Bacteriol 2004;186:7007–7014.

36. Varin T, Lovejoy C, Jungblut AD, Vincent WF, Jacques C. Metagenomic Analysis of Stress Genes in Microbial Mat Communities from Antarctica and the High Arctic. Appl Environ Microbiol. 2012;78:549–559.
37. Jeremy AD, Paul CB, Senthil KM, Wesley DS, Christian AR, Susannah GT, Patrick SGC, Matthew BS, Chien-Chi L, Jason R, Stephen RQ, Brian PH. Single-cell and metagenomic analyses indicate a fermentative and saccharolytic lifestyle for members of the OP9 lineage. Nat Commun. 2013;4:1854.
38. Stephanie AC, Beth NO, Kevin WM, John RS. Abundant Atribacteria in deep marine sediment from the Adélie Basin, Antarctica. Front Microbiol. 2015; 6:872.
39. Dey A, Chattopadhyay A, Mukhopadhyay SK, Saha P, Chatterjee S, Maiti TK, Roy P. Production, Partial Purification and Characterization of an Extracellular Psychrotrophic Lipase from Pseudomonas Sp. ADT3. J Bioremed Biodeg. 2014;5:242.
40. Jain PK, Gupta VK, Gaur RK, Lowry M, Jaroli DP, Chauhan UK. Bioremediation of petroleum oil contaminated soil and water. Res J Environ Toxicol. 2011;5:1-26.
41. Pham VH, Kim J, Jeong SW. Enhanced isolation and culture of highly efficient psychrophilic oildegrading bacteria from oil-contaminated soils in South Korea. J Environ Biol 2014;35:1145-1149.
42. Gupta P, Vakhlu J. Culturable bacterial diversity and hydrolytic enzymes from Drass, a cold desert in India. Afr J Microbiol Res. 2015;9:1866-1876.
43. Cieslinski H, Bialkowskaa A, Tkaczuk K, Dlugolecka A, Kur J, Turkiewicz M. Identification and molecular modeling of a novel lipase from an Antarctic soil metagenomic library. Pol J Microbiol. 2009;58:199–204.
44. Peng Q, Zhang X, Shang M, Wang X, Wang G, Li B, Guan G, Li Y, Wang Y. A novel esterase gene cloned from a metagenomic library from neritic sediments of the South China Sea. Microb Cell Fact 2011;10:95.
45. Berlemont R, Jacquin O, Delsaute M, Salla ML, Georis J, Verte F, Galleni M, Power P. Novel Cold-Adapted Esterase MH lip from an Antarctic Soil metagenome. Biology. 2013;2:177-188.

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Superficial Mycosis in HIV Positive Patients

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Abstract

Introduction: The incidence of superficial mycosis in patients with human immunodeficiency virus (HIV) is increasing in India. *Aims and objectives*: 1. To determine the prevalence, clinical variations, common aetiological agents of superficial mycoses and its CD4 count in HIV-positive subjects in our area. *Methods and Material*: A study was carried out among 150 HIV positive patients over a period of 18 months. Clinical observations were followed by conventional laboratory methods for diagnosis of aetiological fungal species. *Results*: The number of patients 62 / 150 (41.3%) had at least one superficial mycosis. Dermatophytosis (64.71%) was the commonest presentation followed by onychomycosis (26.47%). The two sites involvement was seen in 9.67% followed by (2.94%) patients each with extensive tinea corporis and tinea versicolor. The fungal species isolated were *T. rubrum (35.29%), T. mentagrophyte* (10.29%), *T. verrucosum* (2.94%), *T. tonsurans* (2.94%), *T. schonlenii* (1.47%) and *M. canis* (1.47%). Non-dermatophytes identified were *Candida* spp. (8.82%), *Scopulariopsis* spp. (5.88%), *Fusarium* spp. (1.47%) and *Malassezia* spp. (2.94%). Only one isolate of *Sporothrix sckenskii* was isolated. Statistical analysis : by using statistical package for social sciences (SPSS) software. *Conclusions*: In majority of patients, the clinical presentations did not differ from those found in immunocompetent individuals. We found nondermatophytic onychomycosis and atypical presentations which is rare in immunecompetent patients. We found no increased incidence of superficial mycosis with decreased CD4 count.

Keywords: CD4 Count; HIV; Superficial Mycosis.

Introduction

There is an increased incidence of superficial mycosis among various cutaneous dermatoses, in HIV positive patients. These are extensive, atypical and resistant to the conventional treatment [1,2,3]. also inverse relation with CD4 counts [4]. An Indian study has shown 42% prevalence rate of mycotic skin infections in HIV positive patients [5]. The global variations in the incidence are due to change in climatic conditions, lifestyles, local variations in pathogens and socioeconomic data [6]. Therefore the present study undertaken to know the magnitude of this problem in our area. This will help clinician for correct diagnosis and treatment [7] which improves prognosis.

Material and Methods

The present study was carried out in 150 HIV seropositive patients, attending the ART centre at our tertiary care hospital after the ethical approval from

institutional ethical committee. The clinically diagnosed cases of superficial mycotic infections (involves skin, hair, nail and mucosa) were recruited for this study.

Inclusion Criteria

All HIV positive patients attending ART centre.

Exclusion Criteria

Clinically diagnosed cases of bacterial or viral skin infection, cutaneous drug eruption and systemic mycosis.

Detailed history and informed consent from each patient was recorded in the case report form. The following investigations were done:

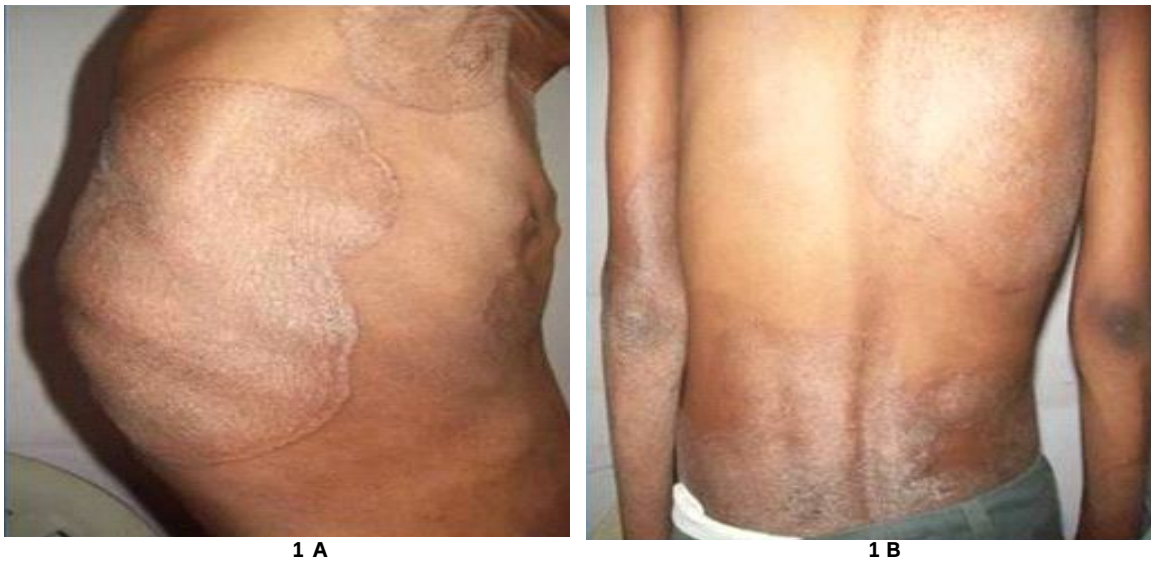
1.CD4 estimation:- By Flow cytometry (BD FACS Caliber micro bead based system.) The most recent CD4 count of the patient was used for analysis.

2. For confirmation of fungal etiology of superficial mycosis: After clinical observations, samples were collected from skin (scraping with scalpel), nails (debris under nail / affected part of nail clipping), hair (by plucking), pus and mucosa (either swab or scraped with blunt scalpel) as appropriate [31]. The sample was used for inoculation of culture media and direct microscopy (10% KOH). (For nail- 20% KOH in sterile bulb overnight) Findings were noted in KOH mount. Fungal culture was done on Sabouraud's dextrose agar (Emmon's modification) with chloramphinicol and DTM (Himedia Pvt Ltd). Incubation, gross examination of fungal colony characteristics and microscopic examination of cultures by lactophenol cotton blue preparation was done as per standard guidelines [31]. Culture with negative reports were repeated in highly suspicious clinical cases as false negative results are known to occur in almost one third of the cultures [3].

In cases where speciation was difficult slide culture was done for mould identification. In addition, tests were done for identification of fungal isolate as dermatophytes which includes thermotolerence tests, urease test**,** healthy hair penetration test, rice grain test. Tests were done for confirmation of thermal dimorphism in case of dimorphic fungi (i.e yeast form and filamentous form of the fungus were demonstrated at two different temperature i.e 25^0C and 37^0C respectively.) The germ tube test was used to identify Candida albicans. In addition to slide culture, Hichrome candida differential agar, sugar assimilation pattern was used for speciation of candida spp. In cases of tinea versicolor, sterile olive oil was added to the culture medium [31].

Observations and Results

A total of (62 / 150) 41% were clinically diagnosed as superficial fungal infection. Of which 51 / 62 (82.18%) were males and 11 (17.82%) were females hence the ratio was 4.6:1. Subjects were between 7-65 years of age hence the mean age was 35.66 + 10.72 years. In our study, (75.8%) men were employed (48.38% in unskilled & 22.58% in skilled) and (4.84%) of females were employed. Most patients 49 / 62 (79.03%) were in early stage while 13 / 62, (20.96%) were in stage IV of HIV infection. The duration of

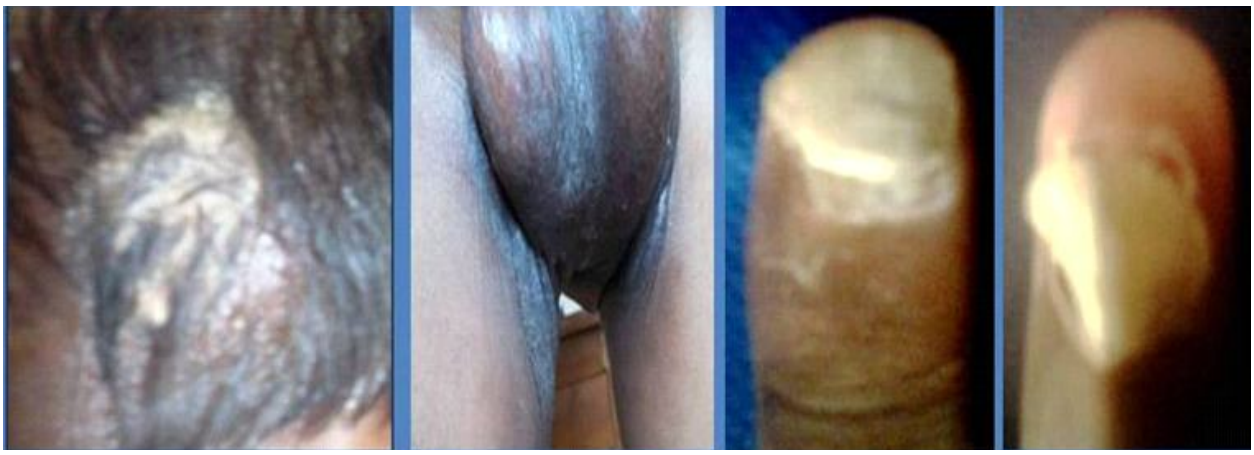


1 A **1 B**

Fig. 1: Extensive Tinea Infection (Fig. 1A,1B: shows involvement >30% body surface area)

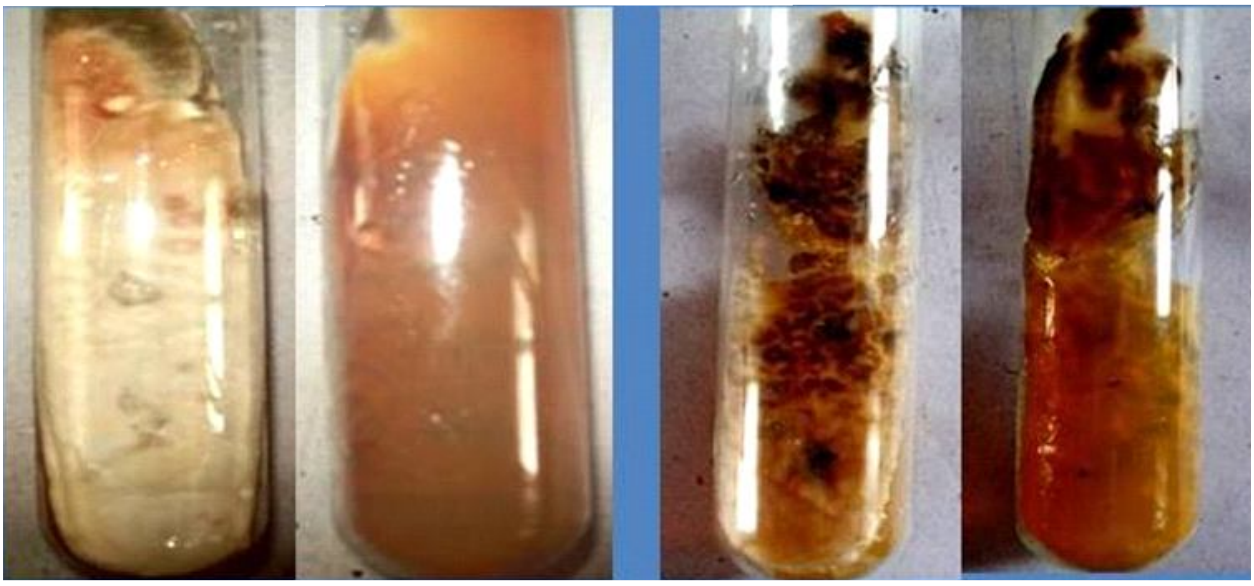
symptoms > one year, six to twelve months, <six months was observed in (41.9%), (22.5%) and (35.4%) patients respectively. There was no predisposing factor such as constant irritation, excessive sweating, trauma etc in majority (45.09%) patients.

Dermatophytosis 44/68 (64.71%) was the most common clinical presentation followed by onychomycosis 32/68 (47%). Out of six (9.67%) two site involvement cases, two patient had toe nail onychomycosis with tinea cruris, one patient had finger nail onychomycosis with tinea pedis, two patient had finger nail onychomycosis with mucocutaneous candidiasis and one patient had tinea corporis with mucocutaneous candidiasis. Cases of extensive tinea corporis (2.94%) (Figure 1), atypical cutaneous sporotrichosis involving right temporal aspect of the scalp in thickened, crusted form with matted hairs which was misdiagnosed as tinea capitis (Figure 2A), superficial mycosis involving external genitalia (scrotum, groin) (Figure 2B), mucocutaneous candidiasis (5.88%) and tinea versicolor (2.94%) were seen. Toe nail and finger nail were affected in 24/68 (35.29%) and 8/68 (11.76%) respectively. The type of most common onychomycosis was Distal and Lateral Superficial Onychomycosis (DLSO) type (81.24%), followed by Proximal Superficial Onychomycosis (PSO) (9.37%) (Figure 2C), Candidial paronychia (6.25%) and Total Dystrophic Onychomycosis (TDO) (3.12%)(Figure 2D).



2 A **2B** **2C** **2D**

Fig. 2: Atypical superficial mycosis and types of onychomycosis **(**Fig. 2A: Shows thick, crusted plaque with matted hairs on Right Temporal area of scalp, Fig. 2B: Shows T. Cruris with involvement of scrotum, Fig. 2C: PSO, Fig. 2D: TDO**)**

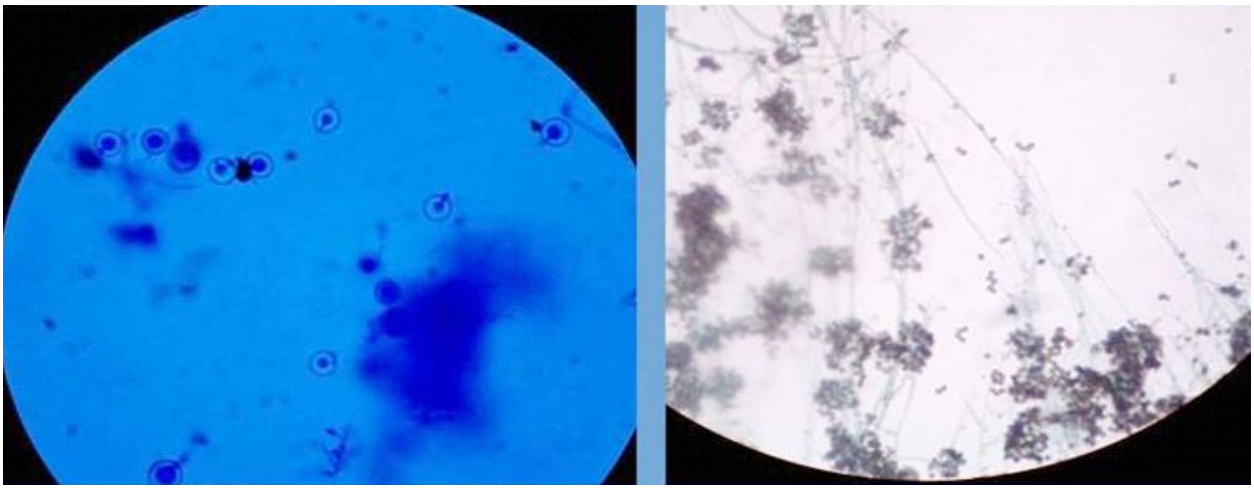


3 A **3B**

Fig. 3: Culture on SDA (non dermatophytes and dimorphic fungi) Fig. 3A: (*Scopulariopsis spp.)* after 25 days Obverse: Whitish to pink, powdery; Reverse: cream to brownish colored. Fig.3B: (*S. schenckii*) after 30 days Obverse: grey brown to black velvety.

Table 1: Involved body site and isolated fungal species (n=68).

Involved body site	Isolated species	No. of isolate	Involved body site	Isolated species	No. of isolate
Skin and mucosa (n=36)	*T. rubrum*	13	Nail (n=32)	*T. rubrum*	11
	T. mentagrophyte	2		*T. mentagrophyte*	5
	T. tonsurans	1		*T. verrucosum*	2
	M. canis	1		*T. tonsurans*	1
	C. albicans	3		*T. schoenlenii*	1
	C. kruzii	1		*C. albicans*	1
	Malassezia spp.	2		*C. kruzii*	1
	Sprothrix schenskii	1		*Scopulariopsis spp.*	4
	No growth	9		*Fusarium spp.*	1
	Contaminants	3		*No growth*	4
				Contaminants	1
		24			27
		Total =	**51/68**		



4 A **4B**

Fig. 4: LCB findings. Fig. 4A: *(Scopulariopsis spp.)* septate hyphae, conidiogenous cell on undifferentiated hyphae. Conidia are one-celled, spherical, 5 x 7µm. Fig. 4B: (*Sporothrix. schenckii*) Slender hyphae, sporulation- 1. Spore on delicate sterigmata along hyphae and 2.pyriform spores in groups; (flower like pattern)

Superficial mycosis involved skin 36/68 (58.08%), nail 32/68 (51.56%) and hair (0%). Six patients had two site involvement i.e 12/68 (17.64%). Dermatophytes 37/68 (54.41%) and non dermatophytes 14/68 (20.58%) were isolated. The KOH mount findings were positive (66.16%) and negative (33.82%). Among all fungal cultures; the results were pathogenic fungi (75%), no growth (19.12%) and contaminated (5.88%). Samples were found positive (60.29%) and negative (16.17%) by both KOH and culture, (17.64%) by culture alone, (5.88%) by KOH alone.

All dermatophytes (n=37)were grown on SDA 37/51 (72.55%) while 29/51 (56.86%) on DTM. The dermatophytes isolated were *T. rubrum* (35.29%) followed by *T. mentagrophyte* (10.29%), *T. verrucosum*(2.94%), *T. tonsurans* (2.94%), *T. schoenlenii* and *M. canis* (1.47%) each. Nondermatophytes isolated were *Candida spp.*(8.82%), *Scopulariopsis spp.* (5.88%) (Figure 3A & 4A), *Fusarium spp.* (1.47%) *and Malassezia spp.* (2.94%). The non-dermatophyte fungi as pathogen had been confirmed by: a) KOH mount Positive 2) Repeated isolation (twice). 3) Immunosupressed state, as used in study by Surjushe Amar et al [12]. Colony morphology on SDA and LCB findings were suggestive of *S. schensckii* (Figure 3B, & 4B) in suspected case of tinea capitis. The findings of samples as culture negative and contaminents were seen in 25% and 5.88% patients.

Mean CD4+ cell count was 288.85/µL. Majority (40.32%) patients had CD4 count between 300 - 700/µl while in (20.96%) patients CD4 count was 201 - 300/µl. The CD4 cell count was between 101 - 200/µL (in 16.12% patients) and 51-100cells/µl (in 11.29% patients). Five subjects (8.06%) had CD4 cell count <50/µL.

Discussion

With the advent of HAART, the course of HIV has changed and also associated dermatological lesions (Maurer and Lori 2004). The prevalence of cutaneous fungal infection was (41.3%) of which 75% cases were confirmed by mycological examination. From India, similar findings had been noted by Kadyada Puttaiah Srikannth et al [5]. (42%). Torssander J et al [8] and Aly R and Tmothy Berger [9] also observed the same (37%).

Majority of our cases of superficial mycosis were seen in the early stage of HIV infection. Kaviarasan PK et al [10] and Rosatelli et al (17% in stage IV HIV patients) and Singh A et al (32.9%), as having similar findings. The age group (21-40 years) predominantly affected. Same age group was affected in study by J Lohoue petmy et al [11] and V Satya Suresh Attili et al [6] Majority of the male (75.8%) and females (4.84%) were employed in unskilled occupation. Kadyada Puttaiah Srikannth et al (48%) **5** and Kaviarasan PK (46%) [10] had also noted same. In our study trauma (20.9%) (in onychomycosis cases) was the commonest predis-posing factor, followed by excessive sweating 12.9%, constant irritation/ occlusion 9.6%, malnutrition 4.8%, continuous immersion of hand in water 3.2% and PVD 1.6%. Surjushe Amar et al [12] had noted trauma 46.66%, Diabetes mellitus 10% and PVD 1.66% as predisposing factors in cases of onychomycosis.

Dermatophytosis

The prevalence of dermatophytosis in our study is 44/150 (29.33%), Kaviarasan PK et al [10] and Di Silverio A at al [13] had similar findings. Kumarasamy N (8%) [2] and Sharma Y K et al (8.33%) [14] noted lower prevalence rate. Tinea unguim (17.74%), tinea corporis (16.12%), tinea cruris (14.72%) was common presentations. Torssander J et al [8] found tinea pedis as the commonest dermatophytosis. Kaviarasan PK et al [10] found 53% tinea corporis, 49% tinea cruris, 7% tinea pedis, and 24.4% tinea ungium cases. These findings showing higher number of patients with mycotic skin infections, may be due to involvement of the HIV stage IV patients [10].

Two case reports of extensive tinea infection (3.22%) was found in our study. D. Craig Wright et al [15] and Goodman DS et al [16] had also reported extensive spreading tinea infection among HIV positive individuals. Study by Kaviarasan PK et al [10] noted 22.8% cases of extensive tinea infection which may be due to HIV group IV study subjects. Dermatophytosis involving penis and scrotum were noted. In a study by Aly R and Tmothy Berger [9]. findings as dermatophytes involving the external genitalia were noted. (a rare site affected in dermatophytosis cases)

Clinically 24/150 (16%) patients were diagnosed with non dermatophytic skin infection. Only a few case reports were available on non dermatophyte causing dermatomycosis [17,18]. The prevalence of onychomycosis was 32/150 (21.33%). We found toe nail (35.29%) and finger nail (11.76%) onychomycosis, similar to the findings of Surjushe Amar et al [12] and Aly R and Tmothy Berger [9]. The frequency, sequence of different types of onychomycosis was same as in study by Surjushe Amar et al [12] and Gupta AK et al [19].

Onychomycosis due to nondermatophytes : Clinical clues used- 1.The absence of T. pedis. 2. Only toe nail affection. 3. History of trauma. 4. non responsiveness to systemic antimycotics. 5. periungual inflammation. Similar clues were used in literature [15]. Majority 18/68 (20.58%) patients had fulfilled the above criteria. Three cases 3/68 (4.41%) of PSO were found in our study. Similar findings were seen in studies by Surjushe Amar et al [12], R Kaur et al [17] and Kaviarasan PK et al [10]. The reason of preponderance of proximal onychomycosis in HIV is unknown [17]. A total of candidial infections 6/68 (8.82%) were seen in our study. The lower number of candidial infections in our study may be due to following reason: 1.The patient selection bias as we have taken patient on ART and not pre ART. 2. OPD rather than IPD patient 3. asymptomatic patients 4. cutaneous *Candidiasis* per se is uncommon.

Tinea versicolor was found in 2/68 (2.94%) cases. No case of tinea versicolor seen in Kadyada Puttaiah Srikannth et al [5]. J Lohoue petmy et al (10.52%) [11], Di Silverio A et al (25%) [13] and D. Craig Wright et al (25%) [15] had seen higher number of tinea versicolor cases. The difference may be due to geographical, climatic variation, asymptomatic patients and HAART. We report a case of cutaneous sporotrichosis 1/68 (1.47%) which was misdiagnosed as tinea capitis. This was an atypical presentation. In India, sporotrichosis is found endemic in few northern states in non HIV populations [26].

T. rubrum (35.29%) was the commonest fungus isolated. This is same as in studies by J Lohoue petmy et al [11] and Kaviarasan PK et al [10]. The other dermatophytes received only a meagre onus of the infections 13/68 (19.11%). Similar findings were noted in the study by Graham E. J. Rodwell et al [20]. We found single case of tinea corporis where *M. canis* was isolated. Bournerias I et al [21] also noted that *M.*

canis was relatively rare even in HIV infected cases. No subject in our study, was infected with *Epidermophyton* spp.. Study by Graham E. J. Rodwell et al [20] also had similar findings. The non dermatophytes were isolated in 14/68 (20.58%); *Scopulariopsis spp., Fusarium spp.*, (& *Candida* spp., *Malassezia* spp.) Similar finding noted by J Lohoue petmy et al [11]. An increasing incidence of non dermatophytic species are on rise in patients with HIV [18]. Speciation holds therapeutic impact [22].

Onychomycosis

We found dermatophytes (62.5%) and non dermatophytes (21.87%) as a causative agent. Ravnborg L et al [23] found dermatophytes more prevalent while Cribier B et al [24] as nondermatophytes. We found *Scopulariopsis* spp. 4/68 (5.88%), *Fusarium* spp. 1/68 (1.47%) as rare isolate. Dompartin D et al [25] had *found 4% Scopulariopsis* spp. And Kedma de Magalhaes Lima [102] and J Lohoue petmy et al[11] had isolated *Fusarium* spp.

Mean CD4 count was 288.85/µl, as in the study by Sharma Y K et al [14] and V Satya Suresh Attili et al [6]. Dermatophytic infections were clustered between 300-700cells/µl. This is in concordance with published literature [27]. The cases of superficial mycosis were not increased with decreased CD4 count. This is also seen in studies by Kumarasamy N et al [2] and Graham E. J. Rodwell et al [20]. Brig Y K Sharma [14] also stated that dermatophytes cannot be taken as AIDS defining illness per se.

Extensive form, atypical presentations and proximal onychomycosis were observed when CD4 count was 50 - 200cells/µl. Out of six cases of fungal infections involving two sites on presentation, two had CD4 counts < 200/µl. Similar findings were observed by Sharma Y K [14] and D. Craig Wright et al [15].

Majority cases of onychomycosis had CD4 count >300 cells/µl. Conant MA et al [28] stated as 450cells/ìl. Tinea versicolor cases were seen between 700 - 1000cells/µl. Patients with cutaneous sporothrix infection had CD4 count 127cells/µl. It was 91 cells/µl by Raquel Vilela et al [29] and 30cells/µl by S. Hardman et al [30].

In the view of these results, emphasis should be placed on the systematic examination of skin in all HIV infected patients. Hence advice regarding the simple preventive measures prevents the recurrence and improves prognosis. This affects the patient's quality of life. Although conventional mycological techniques take a long time (2 - 4 weeks) to identify the fungal isolate, they still hold promise for diagnosis [3,10]. Limitations of the study- there could be subjective differences in interpretation of results but these are minimal. The confirmation of fungal isolate was done with the help of available resources which had been tried to nullify with the help of slide culture. We have not included stage IV HIV patients, hence correlation with CD4 may be affected. We have not done antifungal sensitivity testing for filamentous fungi as the fungi isolated from these group of patients may be resistant to conventional treatment [3]. Hence further research are needed in this arena.

Conclusion

From this study it can be concluded that mycotic skin infections due to dermatophytes were common in HIV positive patients. Atypical presentations such as scrotal and penis involvement by dermatophyte were seen in HIV positive patients. *T. rubrum* was the commonest species. The non dermatophyte especially *Scopulariopsis* spp. as a cause of onychomycosis is an emerging pathogen. Single case reports of infection caused by *Fusarium* spp., *M. canis and S. schenckii* each, points out their emerging pathogenic potential in HIV patients. Most of the fungi causing mycotic skin infections have found as clustered between 100 to 700 cells/µl. This clustering may be due to involvement of only OPD patients on HAART. There was definite correlation between extensive tinea infection, superficial mycosis involving two sites and pathognomic lesion of AIDS such as PSO with CD4 count 50-200/µl.

Key Messages

The presence and importance of Extensive tinea infection, Atypical dermatophytosis, fungal infections involving two sites as well as non-dermatophyte as a causative fungus should be considered in HIV patients as CD4 count decreases.

Abbreviation

DTM: Dermatophyte test medium,

HAART: Highly Active Anti-retroviral Therapy,

HIV: Human Immunodeficiency Virus,

KOH: Potassium Hydroxide,

LCB: Lactophenol cotton blue,

NACO: National

AIDS Control Organization,

NDM: Non Dermatophytic, P

VD: Peripheral Vascular Disease.,

SDA: Sabaroud's Dextrose Agar, Spp.: Species.]

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References

1. Thappa DM. Cutaneous manifestations of HIV infection. In: Valia RG, Valia AR, eds. IADVL textbook of dermatology, 3rd edn. Bhalani publication: 2008;2:1951-1955.
2. Kumarasamy N, Solomon S, Mahivanan P, et al. Dermatologic manifestations among HIV patients in south India. Int J Dermatol. 2000:39:192-5.
3. Marcia Ramos-e-silve, Cinta Maria oliveira Lima, Regina Casz Schechtman Beatriz Moritz Trope, Sueli Carneiro. Superficial mycoses in immunodepressed patients (AIDS). Clinics in Dermatology (2010);28: 217- 225.
4. Bunker CB, Gotch F. AIDS and the skin. In Burns T, Breathnach S, Cox N. et al, eds.Rook's textbook of dermatology, 7th edn. Blackwell publication: 2004;2:26.1-26.41.
5. Kadyada Puttaiah Srikannth, Sunith Vijayakumar, Aparna, Mallikarjun.. A hospital based cross sectional study of mucocutaneous manifestations in HIV infected. International Journal of Collaborative Research on Internal Medicine & Public Health 2010; 2(3):50-78.
6. V Satya Suresh Attili, VP Singh, Shyam Sunder, AK Gulati, DV Sharma, M Rai. Relationship Between Skin Diseases and CD4 Cell Counts in A Hospital based Cohort of HIV infected Adults in North India. Journal of Indian Academy of Clinical Medicine 2008 Jan–March;9(1):20-5.
7. Kannan S, Janaki C, Selvi GS. Prevalence of dermatophytes and other fungal agent isolated from clinical samples. Indian J Med Microbiol. 2006;24: 212- 5.
8. Torssander J, Karlsson A, Manson LM, et al. Dermatophytosis and HIV infection: a study in homosexual men. Acta Derm Venereol. 1998:68;53-6.
9. Aly R, Tmothy Berger. Common superficial fungal infection with AIDS. Clin Infect Dis. 1996;2 Suppl: S128 - 32.
10. Kaviarasan PK, Jaisankar TJ, Thappa DM, S Sujatha. Clinical variations in dermatophytosis in HIV infected patients. IJDVL. 2002;68:213-16.
11. J Lohoue petmy, Lando AJ, Kaptue L, V Tchinda, M Folefack. Superficial mycoses and HIV infection in Yaounde. J European Academy of Dermatology and Venereology. 2004;18:301-304.
12. Amar Surjushe, Ratnakar Kamath, Chetan Oberai, Dattaray Saple, Minal Thakre, Sujata Dharmshale et al. A clinical and mycological study of onychomycosis in HIV infection. Indian J Dermatol Venerol Leprol. 2007 Nov-Dec;73(6):397–401.
13. Di Silverio A, Brazelli V, Brandozzi G, Barbarini G, Maccabruni A, Sacchi S. Prevalence of dermatophytes and yeasts (*Candida* spp.,*Malasezia* furfur) in hiv patients. A study of former drug addict. Mycopathologia, 1991;114(2):103-7.
14. Sharma Y K , Col MPS Sawhne, Lt Col DS Bhakuni, Maj V Gera. Orocutaneous Manifestations as Markers of Disease Progression in HIV Infection in Indian setting. MJAFI 2004;60:239-243.
15. D.Craig Wright, CPT Jeffery L. Lennox, LTC Willium D. James, COL Charles N. Osler, COL Edmond C Tramont. Generalized chronic dermatophytosis in patients with Human immunodeficiency virus type I infection and CD4 depletion. Arch Dermatol, Vol.127, Feb.1991.
16. Goodman DS, Teplitz ED, Wishner A, et al. Prevalence of cutaneous diseases in patients with AIDS/ AIDS related complex. J Am Acad Dermatol. 1987;17:210-20.
17. R Kaur, B Kashyap, P Bhalla. Onycomycosis-epidemiology, diagnosis and management. Indian J Med Microbiol. 2008;26(2):108-116.
18. Kedma de Magalhaes Lima, Celia Maria Machadu Barbosa de castro, Idalina Ines Fonseca Nogueira Cambuim, Jeferson Carvalhaes de oliveira, Marilia Delgado y Rossana sette de Melo Rego. Non-dermatophytic moulds: onycomycosis in four patients infected with human immunodeficiency virus. Rev Iberoam Micol, 2008;25:45-49.
19. Gupta AK, Taborda P, Taborda V, Gilmour J, Rachlis A, Salit L, et al. Epidemiology and prevalence of onychomycosis in HIV positive individuals. Int.J Dermatol. 2000;30:746-53.
20. Graham E.J. Rodwell, Shalene L. Bayles, Loan Towersay, Raza Aly. The prevalence of dermatophyte infection in patients infected with human immunodeficiency virus. InternationaL Journal of Dermatology. 2008;47:339-343.
21. Bournerias I, De Chauvin MF, Datry A, Chambrette I, Carrier J, Devidas A et al. Unusual Microsporum canis infections in adult HIV patients. J Am Acad Dermatol, 1996 Nov;35(5 pt 2):808-10.
22. KD Tripathi. Essentials of medical pharmacology.

Sixth edition, 2009, Jaypee publications.

23. Ravnborg L, Baastrup N, Svejgaard E. Onychomycosis in HIV infected patients. Acta Derm Venerol. 1998; 78:151-2.
24. Cribier B, Mena ML, Rey D, Partisani M, Fabien V, Lang J.M. Nail changes in patients infected with human immunodeficiency virus. A prospective controlled study. Arch Dermatol. 1998;34:216-1226.
25. Dompartin D. Dompartin A, Deluol AM, Grosshans E, Coulaud JP. Onychomycosis in AIDS: clinical and laboratory findings in 62 patients. Int.J Dermatol. 1990; 29:337-9.
26. A.Ghosh, A Chakraborti, V.K.Sharma, K.Singh, A.Singh. Sporotrichosis in Himachal Pradesh (North India).Transaction of the Royal Society of Tropical medicine and hygiene 1999;93(1):S41-45.
27. Current programmes: Dermatological Manifestations in HIV disease. (Adopted from HIV manual 2007). Available from www.27802211.com/programme/programme28.htm. and HIV manual 2007 with Dermatological Manifestations in HIV disease. Available on: http://www.info.gov.hk/aids/pdf/g190htm/iv_index.htm.
28. Conant MA et al.The AIDS epidemic. J Am Acad Dermatol. 1994;31:47-50.
29. Raquel Vilela, Guaenael F. Souza, Glausia Fernandes cota, Loenel Mendosa. Cutaneous and meningeal sporotrichosis in a HIV patient. Rev Iberaom Micol 2007;24:161-163.
30. S.Hardman, I.Stephenson, D.R. Jenkins,M.J.Wiselka, E.M. Johnson. Disseminated *Sporotrhix skenskii* in patient with AIDS. J of infection. 2005;51:e73-e77.
31. Chandra J. chapter In: A Textbook of Medical Mycology. Second edition, Mehta Publishers, New Delhi: 1996.pp.67–79.

Original Article

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Actinomycetic Ulcerative Keratitis

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Abstract

Context: Number of blind people in the world is 45 million. Out of which 5.4 million blind people are in India. Corneal Ulcer is a major cause of blindness throughout the world. About 10% cases of blindness are due to Corneal Ulcer. Corneal Ulcers can be caused by exogenous infections i.e. by viruses, bacteria, fungi or parasites and sometimes it is allergic in nature or it can be due to endogenous infections. Actinomycosis is a rare cause of ulcerative keratitis. *Objective:* To isolate and identify *Actinomyces* from Corneal Ulcer and study their susceptibility and resistance pattern with various antibiotics. *Materials and Methods:* A total of 100 samples were collected during period of June 2014 to March 2015 from ophthalmology hospital, government hospital and clinical laboratories. Samples were collected in sterile container containing 0.5ml of Brain Heart Infusion Broth (BHI) as enrichment culture medium that supports the growth. Isolation of Actinomycetes has been done by using several selective media. Their identification was confirmed by Morphological, Biochemical and Cultural characteristics. After identification *Actinomycetes* species were subjected to antibiotic resistance and sensitivity pattern have been carried out by using disc diffusion technique. *Results:* The results of present study show that Vancomycin is 100% resistant to *Actinomycetes* and Ciprofloxacin, Ofloxacin were effective against *Actinomycetes species* in the treatment of severe Actinomycosis. *Conclusion:* Ciprofloxacin and Ofloxacin for the initial empirical treatment of Actinomycosis is a good option. Prompt diagnosis of corneal ulcers and treatment with appropriate antibiotics prevent blindness and devastating visual disability.

Keywords: Antibiotic Resistance; Corneal Ulcer; *Actinomycetes.*

Introduction

Cornea is a clear transparent front part of the eye with a smooth shining surface. That covers Iris, Pupil and anterior chamber. The cornea with the anterior chamber and lens reflects light with the cornea accounting for approximately two-third of the eye's total optical power. "Corneal Ulcer means loss of corneal substances as a result of infection and formation of raw, excavated area [3]"

Number of blind people in the world is 45 million. Out of which 5.4 million blind people are in India. Corneal Ulcer is a major cause of blindness throughout the world. About 10% cases of blindness are due to Corneal Ulcer [6].

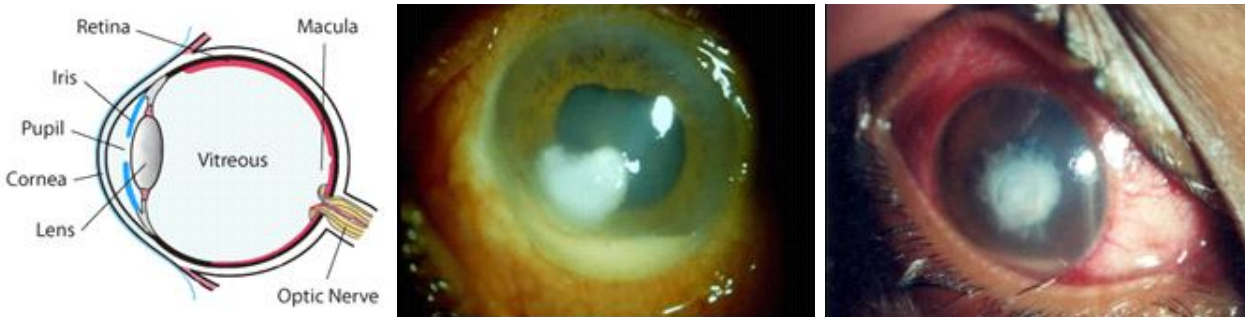


Fig. 1:

Corneal Ulcers can be caused by exogenous infections i.e. by viruses, bacteria, fungi or parasites and sometimes it is allergic in nature or it can be due to endogenous infections. The term keratitis (Corneal Ulcer) had been introduced by "James Wardop" in 1869 in his essay on morbid anatomy of human eye [3,6]. Almost any organism can invade the corneal stroma if the normal corneal defence mechanisms, i.e., lids, tear film and corneal epithelium are compromised [4].

Actinomycosis is a rare cause of ulcerative keratitis. Typical actinomycosis in humans is a chronic disease caused by *Actinomyces spp. Actinomyces* is a genus of the actinobacteria class of bacteria. They are all Gram-positive. Actinomycetes are facultative anaerobic. All species grow best under anaerobic conditions. The infection is characterized by persisting swelling, suppuration, and formation of abscesses with draining sinuses. Major types are cervicofacial, thoracic, and abdominal. In addition to the pathogens in actinomycosis some *Actinomyces* species have also been isolated from other mixed anaerobic infections, eye infections, blood and the urinary tract [2].

Early identification of the organism is essential for effective therapy. The infection normally occurs following trivial corneal injury especially when contaminated by vegetable matter and it is therefore more common in outdoor labourers [7].

Materials and Methods

In assessment to isolate and identify *Actinomyces* from Corneal Ulcer and study their susceptibility and resistance pattern with various antibiotics, present work was under taken.

Collection of Samples

A total of 100 samples were collected during period of June 2014 to March 2015 from ophthalmology hospital, government hospital and clinical laboratories.

Enrichment of Samples

Samples were collected in sterile container containing 0.5ml of Brain Heart Infusion Broth (BHI) as enrichment culture medium that supports the growth of and then transferred immediately to laboratory for further processing [5].

Isolation and Identification of Actinomycetes

After incubation loopful of each enriched culture was streaked on Nutrient agar plates, the plates were incubated at 37°C for 24 hours. Colonies with different morphological characters and Gram's characters were selected and inoculated on respective selective media viz. Brain Heart Infusion agar and Actinomycetes isolation agar. All the plates were incubated at 37°C for 24 hours.

All the suspicious screened colonies of *Actinomycetes* were then analyzed for their biochemical character *viz.* Carbohydrate fermentation, IMViC, Enzymes etc. by inoculating into respective media. Further their identification was confirmed by Morphological, Biochemical and Cultural characteristics.

Antibiotic Resistance Pattern

After identification *Actinomycetes* species were subjected to antibiotic resistance and sensitivity pattern will be carried out by using disc diffusion technique [1].

The Antibiotics were used: Chloramphenicol (30 mcg), Ciprofloxacin (10 mcg), Tetracycline (30 mcg), Gentamicin (10 mcg), Amoxyclav (30 mcg), Cefotaxime (30 mcg), Vancomycin (30mcg), Ofloxacin (2 mcg), Ceftazidime (30mcg), Tobramycin (1.33%).Antibiotic discs were placed on a lawn culture of the isolate under test on Mueller Hinton Agar (MHA).

Positive Amylase Agar | Gram's Staining | Actinomycetes Agar | Positive Urease Agar

Fig. 2:

Result and Discussion

In present study 100 samples were collected during period of June 2014 to March 2015. The patients were of both sex and age groups varying from 20 to 70 years. Out of 100 samples, *Actinomycetes* species were isolated from 24 samples.

Actinomyces is a genus of the actinobacteria class of bacteria. They are all Gram-positive. *Actinomyces* are facultatively anaerobic. All species grow best under anaerobic conditions. *Actinomyces* do not require oxygen for growth and are sometimes referred to as anaerobic bacteria. It is actually the requirement for elevated levels of carbon dioxide rather than the negative effect of oxygen that characterizes *Actinomyces*. Serious ulcers of the cornea of the eye have been caused by contact lens contaminated with saliva containing *Actinomyces*. Among the *Actinomycetes* there are also pathogenic forms which cause Actinomycosis [8].

The *Actinomycetes* were identified based on the colony morphology and biochemical reaction. *Actinomycetes* were confirmed based on light tan colonies onBrain Heart Infusion agar and good luxuriant growth on Actinomycetes isolation agar.

The sensitivity and resistance pattern of*Actinomycetes* against several antibiotics were observed by disc diffusion method on Mueller Hinton Agar (MHA- Hi-media) such as Chloramphenicol (30 mcg), Ciprofloxacin (10 mcg), Tetracycline (30 mcg), Gentamicin (10 mcg), Amoxyclav (30 mcg), Cefotaxime (30 mcg), Vancomycin (30mcg), Ofloxacin (2 mcg), Ceftazidime (30mcg), Tobramycin (1.33%). From these antibiotics *Actinomycetes* showed 70% to 100% resistance against Cefotaxime, Ceftazidime, Vancomycin, Tetracycline, Gentamycin, Amoxyclav antibiotics. These four antibiotics such as Tobramycin, Ciprofloxacin, Ofloxacin, Chloramphenicol showed 75% to 80% sensitivity against *Actinomycetes.* (See Table & Graph No.1)

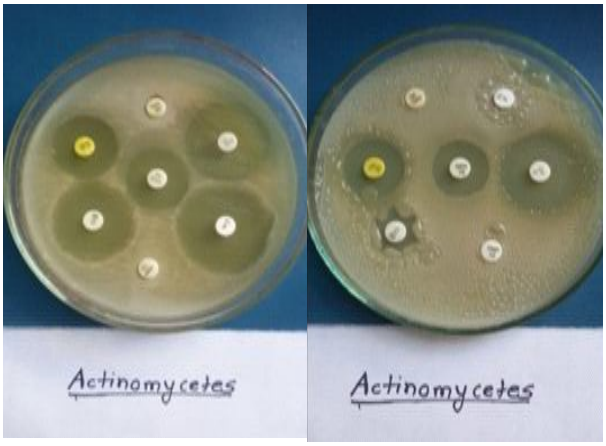
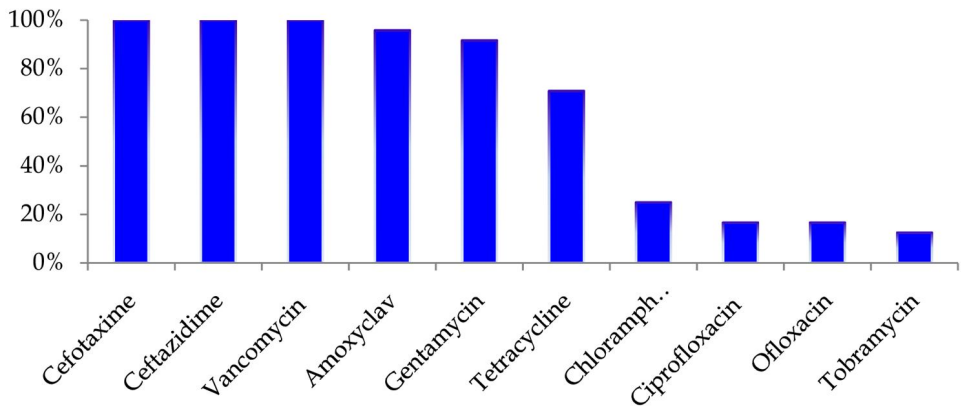


Fig. 3:

Table 1: Resistance and Sensitivity Pattern of *Actinomycetes* against several antibiotics

Sr. No.	Antibiotics	No. of Resistance in Percentage
1.	Cefotaxime	100 %
2.	Ceftazidime	100 %
3.	Vancomycin	100 %
4.	Amoxyclav	95.83 %
5.	Gentamycin	91.66 %
6.	Tetracycline	70.83 %
7.	Chloramphenicol	25.00 %
8.	Ciprofloxacin	16.66 %
9.	Ofloxacin	16.66 %
10.	Tobramycin	12.50 %



Graph 1: Resistance and Sensitivity Pattern of *Actinomycetes* against several antibiotics

Actinomycetes occur in soil. The infection normally occurs following trivial corneal injury especially when contaminated by vegetable matter and it is therefore more common in outdoor labourers [7].

Two genera of the family of Actinomycetacae produce ulcerative lesions of the cornea. Anaerobic Actinomyces is a relatively more common cause of keratoactinomycosis than aerobic Nocardia.A primary Actinomycetic corneal ulcer always follows an injury [7].

Cycloplegic agents such as atropine sulphate 1%, homatropine 1% or cyclopentolate 1% instilled three times a day reduce ciliary spasm and produce mydriasis, thereby relieving pain and preventing synechiae formation [4].

Our results are in accordance with Bharathi *et al.*, 2002 and Bharathi *et al.*, 2010. They observed these antibiotic treatments such as Ciprofloxacin, Ofloxacinwere effective against *Actinomycetes species* in the treatment of severe Actinomycosis. The results of present study show that Vancomycin is 100% resistant to *Actinomycetes* and monotherapy with ciprofloxacin and Ofloxacin for the initial empirical treatment ofActinomycosis is a good option.

Conclusion

Ciprofloxacin and Ofloxacin for the initial empirical treatment of Actinomycosis is a good option. Prompt diagnosis of corneal ulcers and treatment with appropriate antibiotics prevent blindness and devastating visual disability.

References

1. Bauer A W, Kirby W M M, Sherris J C and Turck M. Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 1966;45: 493-6.
2. Bowden Geroge H.W. Medical Microbiology. 4th edition Galveston (TX), Edited by Baron S: University of Texas Medical Branch at Galveston, Chapter 34-Actinomyces, Propionibacterium propionicus, and Streptomyces. 1996.
3. Chatterjee B. M. Hand book of Ophthalmology, Fourth edition, Edited by I.S. Roy, CBS publication, 1988.p.85-113.
4. Garg Prashant , MS and Gullapalli N Rao. Corneal Ulcer: Diagnosis and Management. Community Eye Health, 1999;12(30):21–23.
5. Kaye Stephen B. , Prasad G. Rao, Godfrey Smith, John A. Scott, Sharon Hoyles, Clare E. Morton, Colin Willoughby, Mark Batterbury1and Graham Harvey Simplifying Collection of Corneal Specimens in Cases of Suspected Bacterial Keratitis. J. Clin. Microbiol. 2003;41(7):3192-3197.
6. Ninama Govind L, Jivraj R Damor, Navneet G Padhiyar, Tanuja B Javadekar. to study the causative organism responsiblefor corneal ulcer in S.S.G Hospital Vadodara, Gujarat, National Journal of Community Medicine, 2011;2(2):237-240.
7. Singh Mohinder and Bauit Kaur. Actinomycetic Corneal Ulcer. Eye, 1989;3:460-462.
8. Waksman and S. A. The Actinomycetes, 1959;1(2):61.

Case Series

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Case Series of Ocular Cysticercosis in School Going Children from Foothills of North Himalayan Region of India

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Abstract

Introduction: Ocular cysticercosis is a parasitic infection caused by larvae of Taenia solium. Occurring in 10-30% of the infected patients in endemic areas and may be extraocular or intraocular. It is one of the neglected tropical diseases. It may cause significant visual loss if not treated in time. Here we report three cases of ophthalmic cysticercosis in school going children, all of whom were vegetarian by diet. Each case was unique representing different clinical features and treatment requirements. *Case Series: Case 1:* A five year old school going female presented with painless nodular swelling in her left eye for 3months. On examination, she had a sub conjunctival nodule measuring 7mm x 6mm, about 3mm nasal to the limbus. Her radiological findings revealed a well-defined cystic lesion with well-defined eccentric hyperintensity suggestive of extraocular cysticercosis. Patient underwent surgical excision of the cyst, histopathological examination of which showed features of cysticercosis cellulosae. *Case 2:* A fourteen-year-old male presented with nodular painless swelling in the lateral aspect of the left eye since 8 years. Ocular examination revealed swelling measuring 13mm x 11mm about 6mm temporal to limbus. Clinical and radiological findings were suggestive of sub conjunctival cysticercosis. Patient was taken for surgical excision of the cyst. Histopathological examination revealed cyst of *Taenia solium* confirming the diagnosis of extraocular cysticercosis left eye infiltrating lateral rectus muscle. *Case 3:* An eleven-year-old girl, vegetarian by diet presented with complaints of blurred vision and floaters in right eye for 3 weeks. On retinal examination, a single translucent vitreous cyst was found which on ultrasound scans had features suggestive of intravitreal cysticercosis. Patient was taken up for pars plana vitrectomy for the removal of the cyst, which was removed piecemeal. *Conclusion:* Ocular cysticercosis, a neglected parasitic disease is now-a-days emerging as a common disease in the tropics. The diagnosis is mainly based on clinical features and radiology. Consciousness about importance of early diagnosis, appropriate surgical management and medical management with appropriate anthelminthic drugs should be increased among clinicians with community and personal hygiene measures for prevention of its spread.

Keywords: Ocular Cysticercosis; School-Going Children; Eye Disease.

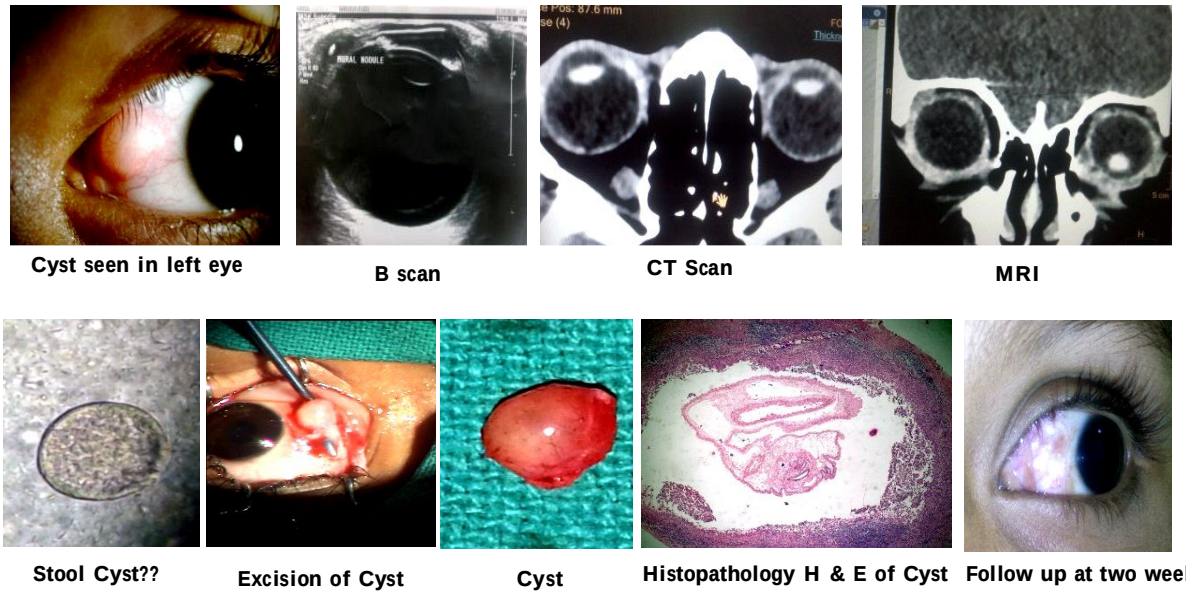
Introduction

Ocular cysticercosis is a parasitic infection caused by larvae of *Taenia solium* (cysticercus cellulosae). This may be acquired by eating undercooked pork (containing larvae) or via ingestion of contaminated water, food and vegetables (containing eggs) or via autoinfection due to unclean personal hygiene [1]. It is one of the neglected tropical diseases prevalent in developing countries like India where both intraocular (subretinal, intravitreal or in the anterior chamber) and extraocular (sub conjunctival or orbital) cysticercosis is observed with almost equal frequency [1,2,3,4]. It may cause significant visual loss if not treated in time, especially if the cyst is located intraocular or if an extraocular cyst compresses on the optic nerve [5,6]. Ocular cysticercosis is usually not the site of primary lesion by this parasite, it is acquired accidently or rarely via dissemination or autoinfection of taenia eggs. Here we report three cases of ophthalmic cysticercosis in school going children, all of whom were vegetarian by diet, from this institute, which caters to patients from foothills of Himalayas lying in Northern India. This region has lot of parasitic infections due to poor hygienic condition, lack of proper sanitation, open defecation and lack of education of proper hand hygiene. School going children are more prone and exposed to these unhygienic food-eating habits. However, few case reports have been there From other parts of India, data from this region of India is not available and actual burden of the disease may still be hidden from the tip of iceberg.

Case Report

Case 1

A five years old school going female child, resident of Nazibabad, Uttarakhand vegetarian by dietary habits, presented with a nodular swelling in her left eye following finger nail trauma 3 months prior to the presentation. The swelling was painless, progressively increasing in size, associated with redness and was being treated outside as conjunctivitis. There were no associated complaints of decrease in vision or double vision, floaters or pain or limitation of ocular movements. There were no neurological signs and symptoms. There was a history of being treated with antitubercular medicines in the past for tubercular lymphadenopathy. On ocular examination her best corrected visual acuity was 6/6 both eyes with a nodular swelling on the bulbar conjunctiva of the left eye measuring 7mm x 6mm, about 3mm nasal to limbus with adherence to the underlying structures. Anterior segment examination on slit lamp biomicroscopy was unremarkable. Although superficial and deep conjunctival and sclera congestion was present, but the conjunctiva overlying the swelling was freely mobile. There was no limitation of ocular motility. Examination of the posterior segment of the eye did not reveal any abnormality. B scan ultrasonography of the mass revealed a well-defined cystic lesion with well-defined eccentric hyperintense mural nodule involving the extraocular adnexal tissue. Computerized tomography showed small well-defined lesion with

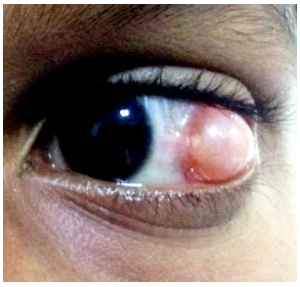


Case 1: Figure

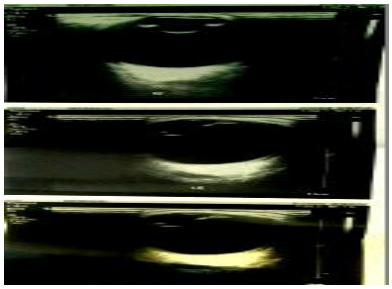
central hyper intensity 5mm x 3 mm in size in both coronal and axial sections involving extraocular tissue on the medial side of the left orbit. Magnetic Resonance imaging showed a cystic lesion with hyperintensity on medial side of the left orbit in T1 weighted images. However, computerized tomography and magnetic resonance imaging of the brain did not reveal any abnormality. Her stool examinations revealed Some cysts, but they were not pathognomic for taenia solium cysts and her ELISA for anti cysticercal antibodies was equivocal. Hence, a preoperative diagnosis of sub conjunctival cyst was made. The patient was taken up for excision of nodular cystic lesion of the left eye under general anaesthesia. Per –operatively it was found that the cyst was infiltrating into the fibers of medial rectus muscle, although ultrasonography did not reveal any such findings. Histopathological examination of the excised specimen showed features of cysticercosis cellulosae with eosinophilia and granulomatous inflammatory reaction in the surrounding tissue, confirming the diagnosis of extraocular cysticercosis of the eye with involvement of extraocular muscle. Post operatively the patient was given a single dose of Albendazole 15 m / kg body weight for deworming. On review, patient's ocular examination was normal and stool examination did not reveal any ova or cyst.

Case 2

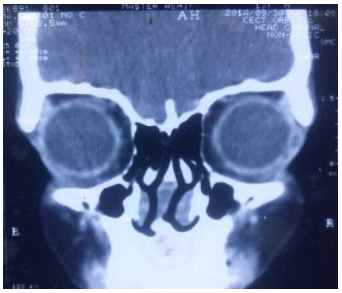
A fourteen-year-old male resident of Bijnour, Uttarakhand presented to the outpatient Ophthalmology department of AIIMS Rishikesh with chief complaints of a nodular painless swelling in the lateral aspect of the left eye since 8 years, which had shown a recent increase in size since last 3 months associated with redness. This was not associated with diminution of vision, diplopia, drooping of lids or protrusion of eyeball. There was no discharge or watering and no evidence of floaters. There was no history of any other systemic illness. Patient was vegetarian in food habits. General physical examination was normal. Ocular examination revealed a nodular swelling measuring 13mm x 11mm about 6mm temporal to limbus with overlying freely mobile conjunctival tissues, but adherence to the underlying structures. Superficial conjunctival and deep scleral congestion was present. Rest of the anterior segment and posterior segment revealed no abnormality. On laboratory investigations ELISA for taenia solium was positive (1.32U) (positive > 1.1 U).Stool findings were negative for any ova or cyst. Ultrasonography B Scan revealed a well-defined extra ocular cystic lesion involving extraocular tissues of the left eye. Was seen.On Computerized Tomography of the orbit, a cystic lesion was seen with peripheral enhancement inthelateralaspect of the left orbit, but computerized tomography of brain did not reveal any evidence of neurocysticercosis.Surgical excision of the cyst was done under general anaesthesiaand the cyst was send for histopathological examination whichrevealed cyst of taenia solium and confirmed the diagnosis of extraocular cysticercosis left eye infiltrating lateral rectus muscle.Oral albendazole was given postoperatively and patient was cosmetically normal and disease free on follow up.



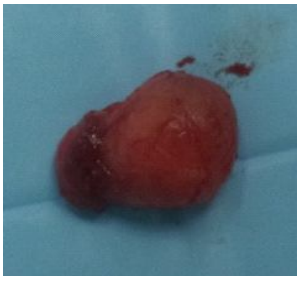
Cyst in eye



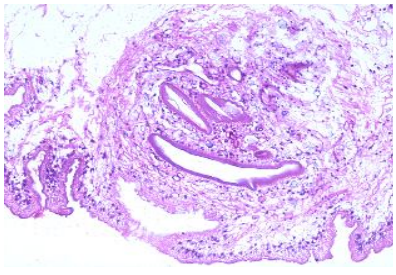
B Scan-USG



CT Scan



Excised Cyst



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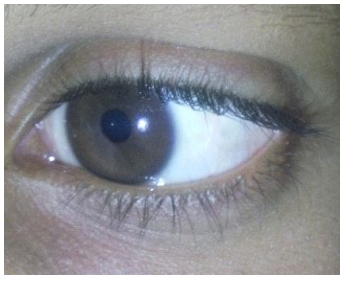
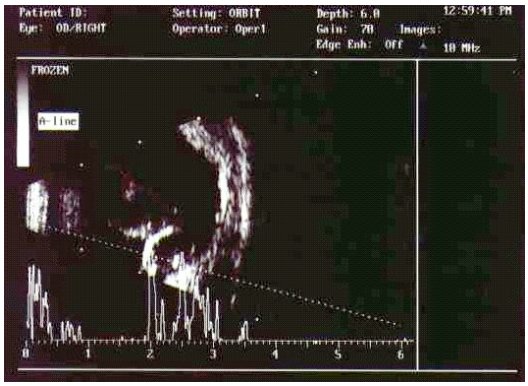


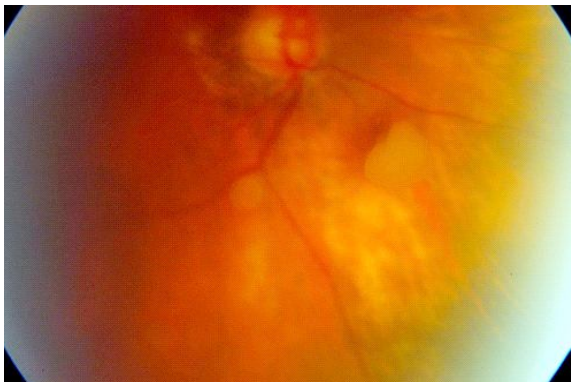
Follow up at 6 weeks

Case 2:

Case 3

An eleven-year-old girl, vegetarian by diet presented to the eye OPD with complaints of blurred vision and floaters in right eye for 3 weeks. She had best corrected visual acuity of 6/24 Right eye and 6/6 left eye. Slit Lamp biomicroscopy of the anterior segment was unremarkable bilaterally. However vitreous examination revealed +1 cells with a floating material in the vitreous cavity. On examination with a fundus viewing lens, a single translucent vitreous cyst was found in the mid vitreous cavity which showed undulating movements within the cyst with light. No other sub retinal cysts were seen. Ultrasound A scan revealed two reflective echo spikes and B scan showed an ovoid echo density in the mid vitreous Blood eosinophilia was noted but stool parasitology examination and ELISA revealed no evidence of cysticercosis. MRI of the brain did not show any evidence of neurocysticercosis. Patient was referred to the vitreoretinal team for pars plana vitrectomy for the removal of the cyst under the cover of systemic steroids. On review post operative examinations patient had a good visual recovery of 6/9.





Case 3: USG scan

Intraviteral cyst on Fundus photograph.

Discussion

Cysticercosis affects an estimated 50 million people worldwide. Ocular cysticercosis is endemic in tropical areas, such as sub-Saharan Africa, India, East Asia, Mexico and Latin America. The reported incidence of ocular involvement varies from 10 to 30% in endemic areas [7]. In India, 78% of the cases with ocular cysticercosis have been reported from states of Andhra Pradesh and Pondicherry (now Puducherry) [8,9]. There is no specific sex predilection. People of any age may be affected, although orbital cysticercosis is more commonly reported in younger age groups. In our case series school going children between 5-15 years of age were diagnosed with ophthalmic cysticercosis. So probably an accidental inoculation may be the cause of such cases. Ocular cysticercosis can involve any part of the eye: approximately 4% involve the eyelid or orbit, 20% involve the subconjunctival space, 8% involve the anterior segment, and 68% involve the posterior segment (subretinal and intravitreal). In Western countries, the most common site of involvement in ocular cysticercosis is subretinal [10].

In India, both intraocular cysticercosis and extraocular cysticercosis are seen with almost equal frequency. Ocular and adnexal cysticercosis represents 13% to 46% of systemic disease. In our series the two cases were myocsticercosis with sub conjunctival presentation and one in vitreous cavity. The presentation of ocular cysticercosis varies based on cyst location, from asymptomatic to vision loss or disturbance and a moving sensation in the eye. Redness, photophobia, and pain may occur. Ocular manifestations may be devastating as the cysticercus enlarges and it may lead to blindness if left untreated [11]. Within the vitreous cavity, the cyst may be free-floating [12,13] and may produce vitritis as was seen in our case 3. Apart from vitritis [14] cysticercosis may also lead to retinal and vitreous hemorrhages, proliferative vitreoretinopathy, retinal detachment, disc edema, cyclitic membrane formation, and phthisis. Cysticerci in the anterior chamber, although uncommon may produce iridocyclitis and secondary glaucoma [15], Conjunctival involvement is usually in the form of a painless or painful yellowish, nodular subconjunctival mass with surrounding conjunctival congestion.

Extraocular myocysticercosis usually presents as recurrent pain, redness, proptosis, ocular motility restriction, diplopia and ptosis [16,17]. One or more extraocular muscles may be simultaneously involved, although a propensity for involvement of the superior

muscle complex and the lateral rectus muscles has been reported [18]. 2 of our patients, although they were cases of myocysticercosis, did not have any diplopia, pain or motility restriction. Also optic nerve compression by an orbital cyst may cause decreased vision and disc edema [19].

In these cases, the presentation was as painless subconjunctival nodules with conjunctival congestion. Although they were myocysticercosis involving medial rectus muscle in one case and lateral rectus muscle in other but presented as subconjunctival nodules due to spontaneous extrusion of cyst from extraocular muscle into the subconjunctival space because of the constant motility of the larvae.

The diagnosis of cysticercosis is based on clinical, serologic, histological and radiological findings. Positive test results from a serum enzyme-linked immunosorbent assay (ELISA) for anticysticercal antibodies help confirm the diagnosis; however, negative test results do not exclude cysticercosis. In fact, only 50% of ocular cysticercosis cases test positive on ELISA, whereas 80% of neurocysticercosis cases test positive [20]. Thus, imaging studies are most helpful in establishing the diagnosis of ocular cysticercosis as was in our case series. High resolution Ultrasonography (USG), computed tomography (CT) and Magnetic Resonance Imaging (MRI) help in detection of the orbital cyst. Though stool examination for the adult worm may be performed in cases of suspected myocysticercosis infections, it is not essential that all patients with myocysticercosis will have the adult worm in their intestines except in those cases, which are acquired by auto-infection. This explains why the stool examination did not show the presence of worms or cyst in our cases.

Surgical removal by pars plana vitrectomy is mandatory in individuals with intraocular cysts. Medical therapy, other than the use of corticosteroids, is not part of treatment. In individuals with uveitis, perioperative corticosteroid administration is recommended. Cysts deep within the orbit are best treated conservatively with a 4-week regimen of oral albendazole (15 mg / kg / d) in conjunction with oral steroids (1.5 mg / kg / d) in a tapering dose over a 1-month period.

The treatment of anterior chamber cysticercosis is essentially surgical. Anterior subconjunctival cysts may be treated with excision biopsy [21] As the cyst is usually adherent to the adjacent muscle, excision may be difficult. Care must be taken to keep the extraocular muscle intact during dissection as was in both of our cases of myocysticercosis.

Conclusion

Ocular cysticercosis, a neglected parasitic disease is now- a-days emerging as a common disease in the tropics. There is a scarcity in sero diagnostics to aid its laboratory diagnosis. The diagnosis is mainly based on clinical features and radiology. Management of both intraocular and extraocular forms continues to pose a serious challenge to the clinicians. Emphasis should be on appropriate sanitation and improvement of personal hygiene to control faeco-oral transmission of the disease. Raw and improperly cooked food should be avoided in endemic areas to reduce the morbidity and ocular blindness due to this preventable disease.

Acknowledgement

Thankful to Dr Jagdish Goyal Additional Professor Department of Paediatrics, AIIMS Jodhpur, who had helped us in preparation of this case series.

Conflict of Interest: NIL

Key Messages

Ocular cysticercosis, a neglected parasitic disease is now- a-days emerging as a common disease in the tropics. There is a scarcity in sero diagnostics to aid its laboratory diagnosis. The diagnosis is mainly based on clinical features and radiology. Management of both intraocular and extraocular forms continues to pose a serious challenge to the clinicians. Emphasis should be on appropriate sanitation and improvement of personal hygiene to control faeco-oral transmission of the disease. Raw and improperly cooked food should be avoided in endemic areas to reduce the morbidity and ocular blindness due to this preventable disease.

References

1. Sanjiv Goyal, Pawandeep Singh Sandhu, Ankita Sharma , Manzoor Ahmad Malik , Parveen Bansal, Jasbir Kaur . Inferior rectus muscle ocular cysticercosis: A case report. Saudi Journal of Ophthalmology 2015; 29:175–177.
2. Murthy GR, Rao AV. Sub-conjunctival cysticercosis. Indian J Ophthalmol 1980;28:77–78.
3. Ziaei M, Elgohary M, Bremner FD. Orbital cysticercosis, case report and review. Orbit 2011;30:230–235.

4. Rath S, Honavar SG, Naik M, Anand R, Agarwal B, Krishnaiah S, et al.Orbital cysticercosis: clinical manifestations, diagnosis, management, and outcome. Ophthalmology 2010;117:600–605.
5. Auzemery A, Andriantsimahavandy A, Esterre P, Bouat C, Boitte JP, Huguet P. Ocular cysticercosis. Med Trop 1995;55(4 Pt 2):429-33.
6. Budke CM, White AC, Garcia HH. Zoonotic Larval Cestodes Infections: Neglected, Neglected Tropical Diseases? PLoS Negl Trop Dis. 2009February;3(2): e319.
7. Pushker N, Bajaj MS, Chandra M. Ocular and orbital cysticercosis. Acta Ophthalmol Scand 2001;79(4): 408–13.
8. Reddy PS, Satyendran OM. Ocular cysticercosis. Am J Ophthalmol 1964;57:664–666.
9. Sen DK, Mathur RN, Thomas A. Ocular cysticercosis in India. Br. J. Ophthalmol 1967;51(9):630–632.
10. Bartholomew RS. Subretinal Cysticercosis. Am J Ophthalmol. 1975;79:670-673.
11. Sharma T, Sinha S, Shah N, et al. Intraocular Cysticercosis: clinical characteristics and visual outcome after vitreoretinal surgery. Ophthalmology May 2003;110(5):996-1004.
12. Wood TR, Binder PS. Intravitreal and intracameral cysticercosis. Ann Ophthalmol. 1979 Jul;11(7): 1033-1036.
13. Seo MS, Woo JM, Park YG. Intravitreal cysticercosis. Korean J Ophthalmol. 1996 Jun;10(1):55-59.
14. Jain IS, Dhir SP, Chattopadhyay PR, Kumar P. Ocular cysticercosis in North India. Indian J Ophthalmol. 1979 Jul;27(2):54-58.
15. Chandra A, Singh MK, Singh VP, et al. A live cysticercosis in anterior chamber leading to glaucoma secondary to pupillary block. J Glaucoma 2007;16(2):271-273.
16. Pandey PK, Chaudhuri Z, Sharma P, Bhomaj S. Extraocular muscle cysticercosis: a clinical masquerade. J. Pediatr Ophthalmol Strabismus 2000;37(5):273–278.
17. Sekhar GC, Lemke BN. Orbital cysticercosis. Ophthalmology 1997;104(10):1599–1604.
18. Sundaram PM, Jayakumar N, Noronha V. Extraocular muscle cysticercosis - a clinical challenge to the ophthalmologists. Orbit 2004;23(4):255-262.
19. J.L.Goyal, S. Das, S. Kumar, D. Chauhan, U. Baheti, V. Sangit. Retrobulbar Cysticercosis Masquerading as Optic Nerve Glioma. Orbit 2007;26(1):61-63.
20. Foster, Stephen; Vitale, Albert. Cysticercosis. Uveitis. 2nd. New Delhi, India: Jaypee-Brothers Medical Publishers; 2013.1290.
21. Honavar SG, Sekhar CG. Ultrasonological characteristics of extraocular cysticercosis. Orbit 1998; 17(4):271-284.

Case Report

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Catheter Related Blood Stream Infection Caused by *Roseomonas Gillardi* in a Neutropenic Patient with Osteosarcoma

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Abstract

Roseomonas gilardii is gram negative pink pigmented non fermentive bacteria and has been responsible for rare cause of human infections. The bacterium considered in recent years to be uncommon, yet potentially of clinical importance as an opportunistic pathogen mostly in patient with underlying disease particularly malignancy. We reported the case of Catheter related blood stream infection in African patient who is on chemotherapy with underlying malignancy. The organism was initially identified by phenotypical characterization and confirmed by 16S RNA gene sequence analysis. Very few such cases had been reported in India.

Keyword: Catheter Related Blood Stream Infection; *Roseomonas Gillardi*.

Introduction

Roseomonas is pink pigmented gram negative, non lactose fermentive coco bacilli. The genus previously classified in to genus *Methylobacterium*. By biochemical reaction and DNA hybridization, 42 strain of pink pigmented gram negative bacteria had been separated from genus *Methylobacterium* and new group has been introduces as "pink coccoid"[1]. The majority clinical isolate were from blood. We are presenting the case catheter related blood stream infection due to *Roseomonas gillardi* in 19 year male patient form Nairobi suffering from Osteosarcoma of iliac bone and he was on chemotherapy.

Case Report

A 19 year male patient from Nairobi was referred to our oncology department with having 3 month history of dull ache in the right pelvic region. CAT scan of the pelvis showed a right iliac bone mass characteristic of Osteosarcoma. Open biopsy were performed and diagnosed as Osteosarcoma of right iliac bone. PET scan has been done and suggestive of non metastatic. Wide excision has been planned, performed and Implant has been fixed. Patient was ifosfamide and etoposide chemotherapy. After the second cycle of Chemotherphy, patient developed high grade fever. His investigation showed Hamoglobin of 8.5 gm%, Total leucocyte count of 2640 cells/microliter, Differential count- Neutriophil 52%, Lymphocyte 30%, Eosinophil 02%, Monocyte 15%, Platelet count of 3,15,000 cells/microliter and Procalcitonin 1.15ng/ml. Febrile illness with leucopenia and high procalcitonin were suspicious of CRBSI (Catheter related Blood stream infection). Blood was withdrawn for two blood culture set, from peripheral vein and from port line and inoculated each in to Bactec Plus Aerobic (10ml), Bactec Plus Anaerobic(10 ml) and Bactec Myco-F bottle (5ml). The port line Plus Aerobic and Myco-F bottle beeped positive at 34 and 35 hours of incubation respectively.

Peripheral vein Plus Aerobic and Myco-F bottle were beeped positive at 40 and 44 hours of incubation respectively. Sub culture from positive bottle done on Sheep Blood Agar, MacConkey agar, Neutrient Agar and Chocoloate agar. Neutrient agar showed pink colored colony (Figure 1) with in 24 hours of incubation at 37^{0}C. Species identification was done on Vitek 2 and identified as *Roseomonas gilardii.* As it is unusual organism, colony further processed for bacterial 16S rRNA gene sequence analysis for confirming the phenotypical method of identification by genotypic method. The identified sequence were queried to library Sepsitest™ BLAST, Version 0.9, DB rel. 95.2 for match and BLAST hit with highest sequence identity as, Species *Roseomonas gilardii,* Sequence identity = 99.0%; E-Value = 0.0; Accession = AY150045. Antimicrobial susceptibility showed sensitive to Ampiclin (MIC ≤ 2) Ceftriaxone (MIC ≤ 1), Cefipime(MIC≤4), Imipenem(MIC≤0.25), Meropenem(MIC≤0.25), Amikacin(MIC≤2), Gentamicin(MIC≤ 1), Ciprofloxacin(MIC ≤ 0.25) and Tigecycline(MIC≤ 0.5). Cefoperazone + Sulbactum was intermediate (MIC 32).

After having culture report, Port has been removed. Patient treated with Imipenem 1gm 8 hourly and Amikacin 10mg/kg/day. After the third day of antimicrobial therapy, patient became afebrile. Antimicrobial therpy were continued for 10 days. Post antimicrobial therapy, repeat blood culture has been taken twice with one week gap and all were reported as sterile.

Fig. 1:

Discussion

Roseomonas gilardii is a gram-negative coccobacilli having pink colored colonies and belonging to the genus *Roseomonas.* This bacteria consider to be environmental bacteria and have been reported from clinical specimens for the last 4 decades. Initially this group had been referred to as Pink Coccoid groups[2,3]. Rihs et al has worked on 42 isolates of pink pigmented bacteria and classified genus into six *Roseomonas* species, 1 to 4 based on biochemical reaction and 5 to 6 based on DNA hybridization techniques. Of these six, *R. gilardii* is most frequently related to human infections.

Roseomonas spp. considered as opportunistic pathogen with low pathogenic potential for humans, but some species may cause clinically significant or even fatal disease in immunocompromised patients. The natural reservoir for this infection is not known but contaminated water could be the major source [3]. According to literature of *R. gilardii* infections in majority of cases, the initial symptoms were suggestive of bacteremia and associated with the presence of a central line [1]. Other sites of infection have also been reported; respiratory infection, wound infection, osteomyelitis, peritonitis and eye infection [1,4,5]. Underlying disease like, malignancy (most reported), renal disease, inflammatory bowel disease and diabetes are common finding with this infection [1,4,5]. The biofilm production on catheter may be playing an important role in the virulence of invasive infections due to species with a 'mucoid in nature'[6].

The choice of an effective drug for empirical treatment of infections due to *Roseomonas spp*. is sometimes difficult. According to the results of a recent review (De´ et al., 2004), the most active agents against *Roseomonas* species are amikacin and imipenem (99 % susceptibility), followed by ciprofloxacin (90%) and ticarcillin (83 %). Conversely, antibiotics such as third- or fourth-generation cephalosporins are not appropriate for treating infections due to this organism. Susceptibility varies among the different species;

The choice of an effective empirical antimicrobial therapy to the infections caused by *Roseomonas spp.* is sometimes difficult. According to the literature review *R. mucosa* has the higher risk of resistance whereas *R. gilardii* strains are the most susceptible. Rihs et al found that all six species *Roseomonas* genus exhibited >96% resistance to cephalosporins [1]. The most active agents against *Roseomonas* species are Aminoglycoside and Carabapenam. Susceptibility varies among the different species [7].

Conclusion

In conclusion, our case underlines the clinical significance of *Roseomonas* species, particularly in the presence of an indwelling catheter device in patient with malignancy. Microbiologist and Clinician should familiarize themselves with the characteristics of infection with *R. gilardii* because of diagnostic and management implications. Molecular typing by 16S

rRNA gene sequence helps in confirming the phonotypicaly identified strain. It also helps in epidemiological investigation for identifying the source in case of outbreaks. Differences in susceptibility patterns and virulence among the various species highlight the importance of definite identification of *Roseomonas* isolates. Broad-spectrum antibiotics Carbapenems and possibly combination therapy (including an aminoglycoside / quinolones) should be the first choice for the empirical treatment of *Roseomonas* infections and Cephalosporin should be avoided.

References

1. Rihs, J. D., D. J. Brenner, R. E. Weaver, A. G. Steigerwalt, D. G. Hollis, and V. L. Yu. *Roseomonas*, a new genus associated with bacteremia and other human infections. Journal Clin Microbiol 1993;31:3275-83.
2. Gilardi, G. L., and Y. C. Faur**.** *Pseudomonas mesophilica* and an unnamed taxon, clinical isolates of pink-pigmented oxidative bacteria. Journal Clin Microbiol 1984;20:626-9.
3. Korvick, J. A., J. D. Rihs, G. L. Gilardi, and V. L. Yu**.** A pink-pigmented, oxidative nonmotile bacterium as a cause of opportunistic infections. Arch Intern Med 1989;149:1449-51.
4. Struthers, M., J. Wong, and J. M. Janda. An initial appraisal of the clinical significance of *Roseomonas* species associated with human infections. Clin Infect Dis 1996;23:729-33.
5. Sandoe, J. A. T., H. Malnick, and K. W. Loudon. A case of peritonitis caused by *Roseomonas gilardii* in a patient undergoing continuous ambulatory peritoneal dialysis. Journal Clin Microbiol 1997;35:2150-2.
6. Elshibly, S., Xu, J., McClurg, R. B., Rooney, P. J., Millar, B. C., Alexander, H. D., Kettle, P. & Moore, J. E. Central line-related bacteremia due to *Roseomonas* mucosa in a patient with diffuse large B-cell non-Hodgkin's lymphoma. Leuk Lymphoma 2005;46;611–4.
7. De´ , I., Rolston, K. V. I. & Han, X. Y. Clinical significance of *Roseomonas* species isolated from catheter and blood samples: analysis of 36 cases in patients with cancer. Clin Infect Dis 2004;38:1579–84.

STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS

"Journal of Microbiology and Related Research" (See Rule 8)

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Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors.

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Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

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1) Type of manuscript (e.g. Original article, Review article, Case Report)
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The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

Introduction

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http://www.consort-statement.org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/l7-c_e.html).

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Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

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Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

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- Conflicts of interest disclosed

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- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
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- References according to the journal's instructions

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- Uniformly American English
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- Labels pasted on back of the photographs (no names written)
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