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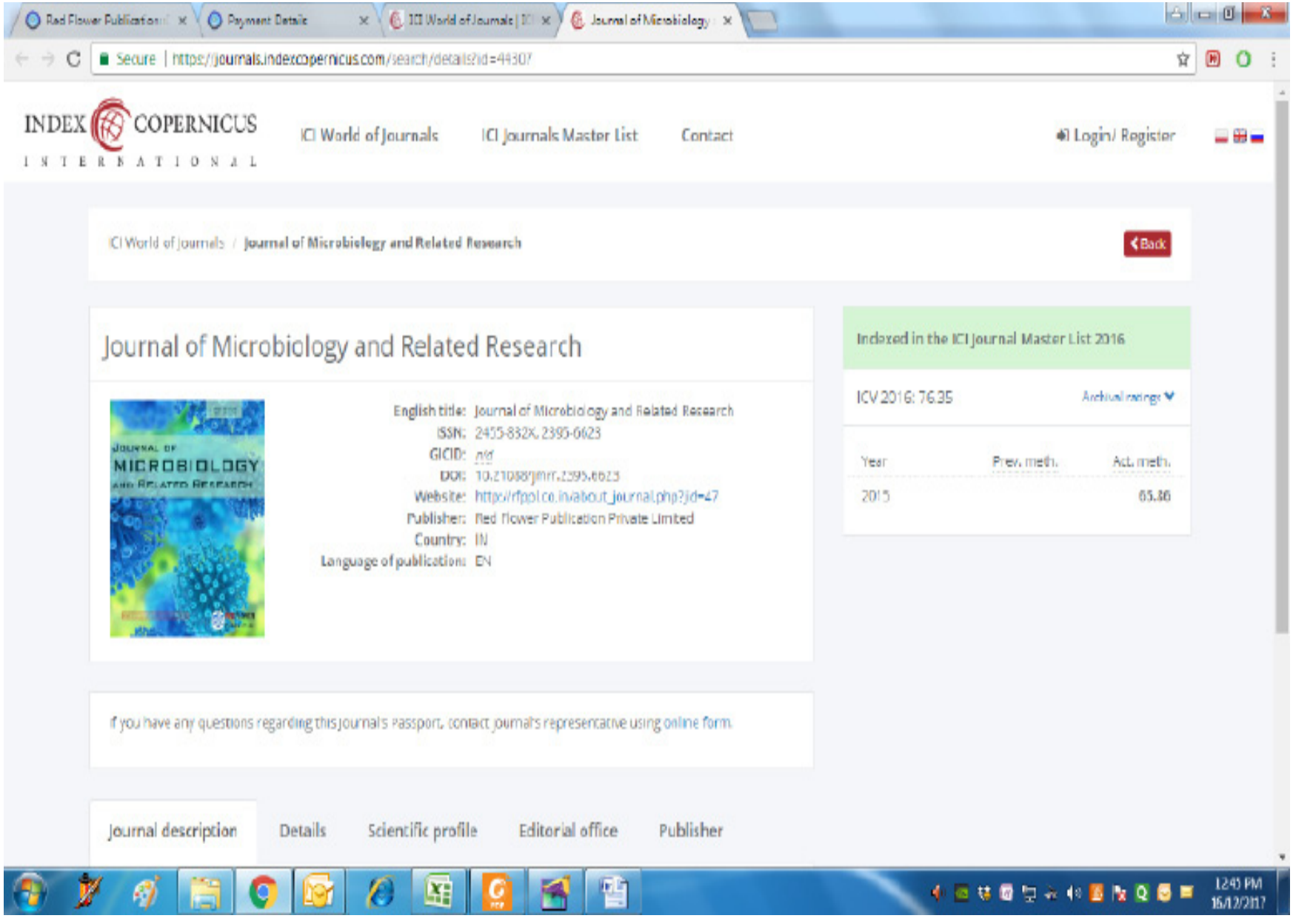
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Prevalence and detection of co-existence of multiple β lactamases in clinical isolates: A diagnosticchallenge and a dangerous trend

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Abstract

Introduction: The greatest threat that diagnosticians and physicians face today is from the multi drug resistant bacteria. The multi-drug resistance associated with extended spectrum β lactamases (ESBL), Amp-C and metallo β lactamase (MBL) producers poses a considerable therapeutic challenge leading to treatment failure. So the present study was conducted to detect the occurrence of various β Lactamases in the clinical isolates. *Materials and Methods:* We included a total of consecutive 206 isolates of Escherichia coli and Klebsiella pneumonia in our study, over a period of one year. Antibiotic susceptibility testing was performed in accordance with guidelines by Clinical and Laboratory Standards institute (CLSI). ESBL production was confirmed by using the double disc diffusion test recommended by CLSI. AmpC disc test was performed for confirmation of AmpC production and MBL producers were detected using EDTA disc potentiation test. *Result:* Of the total 206 isolates, 46 (22.33%) were found to be pure ESBL producers, 64 (31.06) were AmpC producers out of which only one isolate produced pure AmpC. MBL production was confirmed in 11 (5.33%) isolates. The significant finding of this study was the co production of ESBL and AmpC which was detected in 53 (25.72%), taking the total number of ESBL producers to 99 (48.05%). AmpC and MBL were co produced by 10 (4.85%) isolates. Conclusion: Routine laboratory detection of multiple beta lactamases has become the need of the hour and is recommended, since the occurrence and co-existence of beta lactamases in Gram negative bacilli is on the rise.

Keywords: Extended Spectrum Beta Lactamases (ESBL); AmpC Beta Lactamase; Metallo Beta Lactamase (MBL); Co-Producers.

Introduction

β-lactam antibiotics are among the most frequently prescribed antibiotics worldwide [1]. Among the variety of mechanisms that can provide resistance to β-lactam antibiotics in Gram-negative bacilli, the production of β-lactamase is by far the single most important factor [1]. Extended spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo β-lactamases (MBLs) are the major causes of β-lactam resistance in Gram negative bacteria [2].

The ESBL enzymes are beta-lactamases capable of conferring bacterial resistance to the penicillins; first-, second- and third-generation cephalosporins; and aztreonam (but not the cephamycins or

carbapenems), and which are inhibited by beta-lactamase inhibitors such as clavulanic acid [3]. AmpC beta-lactamases confer resistance to a wide variety of β-lactam drugs, including α-methoxy-β-lactams, such as cefoxitin, narrow, expanded-, and broad-spectrum cephalosporins, β-lactam-beta lactamase inhibitor combinations, and aztreonam. Metallo beta-lactamases (MBLs) are the Ambler class B beta-lactamases which have the capacity to hydrolyze with the exception of aztreonam, all beta-lactams including carbapenems [4].

To detect resistance to various ß-lactam group drugs via ESBLs, AmpC, and MBL, various phenotypic tests can be used. However, phenotypic testing may be misleading, especially when both ESBL and AmpC ß-lactamases co-exist and mask each other, which results in misreporting and failure in clinical treatment of patients, while the presence of both MBLs and AmpC ß-lactamases in a isolate confers carbapenem resistance [5]. In view of the increasing significance of coexistence of β-lactamases, the present study was undertaken to know the prevalence of coexistence of ESBLs, AmpC β-lactamases and MBL in clinical isolates of *Escherichia coli* and *Klebsiellapneumoniae* by using different phenotypic methods.

Materials and Methods

The present study which aimed at detecting the prevalence of Co-producers of beta lactamases, was carried out in Department of Microbiology, at a tertiary care hospital in Karnataka over a period of one year from December 2013 to December 2014. A total of 206 consecutive isolates of *Escherichia coli* and *Klebsiellapneumoniae* together which were isolated from various clinical samples like pus, blood, body fluids, urine etc were included in this study. Standard microbiological procedures were followed in identification of the isolates and their Antibiotic susceptibility testing was carried out by Kirby Baeur method as per the Clinical and Laboratory StandardInstitute (CLSI) criteria [6].

ESBL detection [6,3]

A zone diameter of <22mm for Ceftazidime 30µg disk was taken as Screening test positive for ESBL. All *E.coli* and *Klebsiellapneumoniae* which were screening positive were subjected to phenotypic confirmatory tests for ESBL detection.

Phenotypic confirmation test for ESBL detection [6,3]: Lawn culture of the isolate was made. Ceftazidime disks (30µg) alone and in combination with clavulanate (10 µg) were placed at a distance of 20 mm and incubated for 24 hours at 37°C. A difference of ≥5 mm between the zone diameters of ceftazidime disk and the ceftazidime-clavulanate combination disk was taken to be confirmatory for ESBL production.

Amp-C production [7]: Isolates were screened for Amp-C production by using Cefoxitin disk (30µg). Isolates with cefoxitin zone of <18 mm were considered as screen positives for Amp-C beta-lactamase production.

Phenotypic confirmatory test: AmpC disk test [7]: A lawn culture of Escherichia coli ATCC 25922 was prepared on MHA plate. Sterile disks (6mm) moistened with sterile saline (20 µl) were taken and inoculated with several colonies of the test organism. The inoculated disk was then placed beside a Cefoxitin disk (almost touching) on the inoculated plate. The plates were incubated overnight at 37^0 C.A positive test appears as a flattening or indentation of the cefoxitininhibition zone in the vicinity of the test disk. An undistorted zone of inhibition is considered as a negative test.

MBL production: The isolates exhibiting resistance to imipenem were suspected of MBL production. MBL production among them was confirmed byImipenem-EDTA disc test. Two 10 µgimipenem discs were placed on the lawn cultured MHA plate of test organism. 5µL of sterile 0.5 ml solution of EDTA was added on one of the disc.The inhibition zones of imipenem-EDTA discs were compared after 16-18 hours incubation at 35°C. An increase in the zone size of atleast 7mm around the imipenem-EDTA disc compared to plain imipenem disc was recorded as an MBL- positive strain [8].

Results

99 (48.05%) Gram negative clinical isolates were screened positive for ESBL production out of the total 206 clinical isolates processed. Confirmatory test confirmed the production of pureESBL in 46 (22.33%) isolates. Among the 206 isolates, 76 (36.89%) isolates were found to be resistant to cefoxitin (Screening test positive for AmpC). AmpC disk test confirmed the production of AmpC in only 64 (31.06%) isolates.16 (7.76%) isolates were screening test positive for MBL and MBL production was confirmed in 11 (5.33%) isolates using EDTA disc potentiation test. Co-production

of ESBL and AmpC was found in 53 (25.72%) clinical isolates.AmpC and MBL co-occurred in 10 (4.85%) organisms. The remaining 94 (45.63%) isolates were not found to harbour any of the three enzymes. Table 1 shows the distribution of different beta lactamases in the isolated organisms. Chart 1 depicts the antibiotic resistance patterns of beta lactamase produers and non producers.

Discussion

The incidence of infections due to organisms resistant to β lactam agents has increased sharply in recent years [9]. This increasing incidence of multiple β lactamase producing organisms all over the world holds even more significance because organisms frequently also possess resistance factors to other classes of antibiotics, notably the aminoglycosides and fluoroquinolones [10]. Most of the laboratories do not consistently test for β lactamases [11], and this can be detrimental to the patient who is treated with a cephalosporin in the mistaken belief that the isolate is susceptible [12]. The present study was conducted to detect the prevalence of Co-producers of beta lactamases and the challenges faced in detecting them using phenotypic tests.

In the present study, 46 (22.33%) isolates were confirmed to produce pure ESBL, of which 26 (12.62%) were E.coli and 20 (9.70%) were Klebsiellapneumoniae. In the western studies conducted by Lautenbach et al. [9], Ozgunes et al. [13], Schwaber et al. [14] and Lee Young et al. [15], *K.pneumoniae*was the commonest organism found. Thus our study performed on Indian population did not correlate with the above four western studies. In another Indian study conducted by Shanthi M and Uma Sekar [16] at Chennai, *E.coli* was found to be the predominant ESBL producing isolate, accounting for 72.02%, followed by Klebsiella. The predominance of ESBL positive *E.coli*in the study by Shanthi M et al. [16] and our study points

Table 1: Organism wise distribution of ESBL, AmpC, MBL and co-production.

Organism/ β-lactamase	*Escherichia coli*	*Klebsiellapneumoniae*	*Total*
Pure ESBL	26 (12.62%)	20 (9.70%)	46 (22.33%)
Pure AmpC	01 (0.48%)	0	01 (0.48%)
Pure MBL	0	01 (0.48%)	01 (0.48%)
ESBL + AmpC	32 (15.53%)	21 (10.19%)	53 (25.72%)
AmpC + MBL	05 (2.42%)	05 (2.42%)	10 (4.85%)
ESBL + MBL	0	0	0
Non producers	54 (26.21%)	41 (19.90%)	95 (46.11%)
Total	118 (57.28%)	88 (42.71%)	206 (100%)

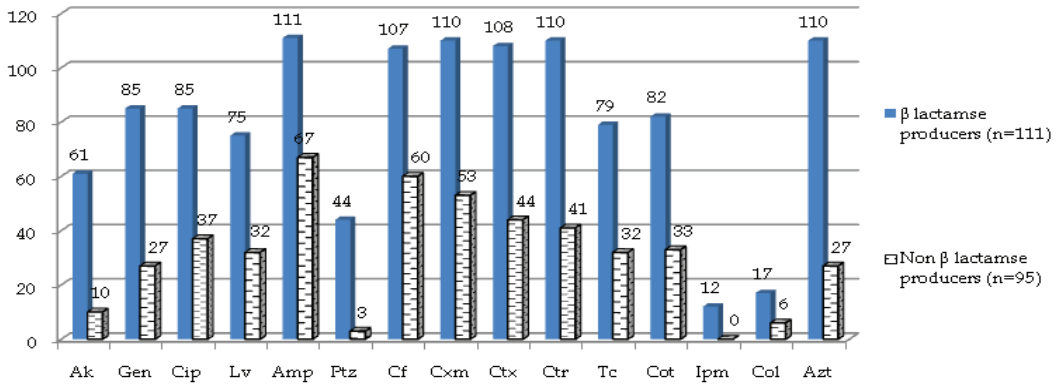

Chart 1: Antibiotic resistance patterns of beta lactamase producers and non producers

Index: Ak=amikacin, Gen=gentamicin, Cip=ciprofloxacin, Lv=levofloxacin, Amp=amoicillin, Ptz=piperacillin-tazobactam, Cf=cephalothin, Cxm=cefuroxime, ctx=cefotaxime, ctr=ceftriaxone, Tc=tetracycline, Cot=cotrimoxazole, Ipm=imipenem, Col=colistin,Azt=aztreonam.

towards the predominance of the isolate in this geographical location as compared to the western reports. The present study detected presence of AmpC β lactamase in 64 (31.06%) isolates. Other studies have shown AmpC occurrence varying from 14.8% - 52.1% [17-20]

Since the incidence of co producers of ESBL and AmpC is rising through out the world, we faced certain challenges in detection of these co producers. The inhibitor-based confirmatory test approach for ESBL detection is most reliable for isolates that do not coproduce an inhibitor resistant beta-lactamase, such as AmpC. High level expression of AmpC may prevent recognition of an ESBL. This problem is more common in tests with species or strains that produce a chromosomally encoded inducible AmpC beta-lactamase. With these organisms, clavulanate may act as an inducer of high level AmpC production and increase the resistance of the isolate to other screening drugs, producing a false negative result in the ESBL detection test [21]. Since the co-producers produced both ESBL and AmpC beta lactamase enzymes, the confirmatory test for ESBL detection by Cephalosporin/clavulanate combination disk test could not be interpreted correctly. However studies have indicated that AmpC has minimal effect on activity of cefepime, making it a more reliable indicator of ESBL production in presence of AmpC [22]. So the susceptibility pattern of all AmpC producers to cefepime was noted down in this study and it was found that, out of total 64 Amp C producers, 53 (25.72%) isolates were resistant to cefepime. This indicated 53 isolates were co producers of ESBL and Amp C, making the total number of ESBL producers 99 (48.05%). Other confirmatory tests using Tazobactam and sulbactam as inhibitors instead of clavulanate,are proven to be more reliable in ESBL detection in presence of AmpC [21].

In our study, only one isolate was found to produce pure AmpC β lactamase, while one isolate was detected to produce pure MBL. 10 (4.85%) isolates were found to co produce AmpC and MBL. Various studies have documented the incidence of multiple beta lactamase production ranging from 1.33% to 42.75% [17-20]. The reason for wide range in incidences could be factors like different antibiotic usage pattern leading to gene mutations or difference in normal flora depending upon cultural, nutritional or ethnic variations in different populations. The major cause of high incidence of ESBL and Amp C coproduction is inappropriate use of extended spectrum cephalosporins [10].

The majority of ESBL and AmpC producing organisms were found to exhibit high rates of resistance to commonly used antibiotics as shown in chart 1. Specifically, high rates of resistance observed to fluoroquinolones (76.57% resistance to ciprofloxacin, 67.56% resistance to levofloxacin) and cotrimoxazole (73.87% resistance) are most worrisome, as these cost effective drugs are most widely used. The results of present study were correlating with studies done by Baral P et al [23], Tumbarello M et al. [24] and Ozgunes I et al [13]. All the above studies showed that ESBL and AmpC producing organisms had a multidrug resistance potential. This might be due to the fact that ESBL genes are usually found in large plasmids that also contain other antimicrobial resistance genes, and hence ESBL-producing organisms may also show resistance to aminoglycosides, tetracyclines, chloramphenicol and/or aminoglycosides [14]. Another reason for such multidrug resistance potential of ESBL producers might be due to selective antibiotic pressure in the environment.

Conclusion

High degree of antibiotic co-resistance among beta lactamase producers emphasizes the judicious use of antimicrobials. Imipenem still remains most effective drug against ESBL and AmpC producing organisms followed by piperacillin-tazobactam [25]. Multiple beta lactamase detection and drug susceptibility can be routinely undertaken to enable the clinicians to select appropriate antibiotics [26]. Besides, formulating and adhering to an antibiotic policy is imperative to prevent selection pressure & spread of multidrug resistance strains.

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Original Article

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Aerobic bacteriological profile of post operative wound infection in tertiary care hospital, Bhopal

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Abstract

Surgical wound infections remains one of the most important post operative complications accounting for 10 to 20% of the hospital cost. In most post operative SSI the causative pathogens originates from normal flora of patients skin, mucous membrane or hollow viscera. SSI can cause considerable mortality and morbidity. Wide spread use of antibiotics leads to major problem of MDR organisms contributing to longer stay in hospitals. Hence the present study was carried out to determine the incidence of surgical wound infection with antibiogram.

Introduction

Wound infection are one of the most common hospital acquired infections causing considerable morbidity mortality [1].

The sepsis in modern surgery continuous to be the significant problem for health care practitioners across the globe [2].

Surgical wound infection remains one of the most important post operative complications, accounting for 10 to 20 % of the hospital cost [3].

Despite the advances made in asepsis, anti microbial drugs, sterilization and operative techniques. Surgical site infection continues to be major problems in all branches of surgery in hospital [4].

The common causative agents are Staphylococcus aureus, pseudomonas aeruginosa, Escherchia coli and klebsiella [5].

Complicated surgical procedures have a grave impact increasing duration of hospitalization and cost of hospitalization [6].

Wide-spread use of antibiotics leads to major problem of multi drug resistance organism contributing to morbidity and mortality [7].

The center for disease control and prevention (CDC) had pointed out that, " The most important measure for preventing the spread of nosocomial bacterial pathogens is effective hand washing". Most guideline recommends hand washing before and after contact with patients, before invasive procedure and after contact with contaminated inanimate objects (Garner et al,1996) [8].

After the general use of modern antibiotic therapy for 60 years, clinical experience and bacteriological studies have shown that the overall incidence of infection in the surgical patient has not decreased and many related problems are still present. This is because we have a wide variety of surgical procedures to treat different conditions, which were not available 10 to 20 years ago. Advanced medical science, has given the knowledge to practice the various preventive measures to control the routinely encountered types of infection, but other types of infection have taken their place [9].

Wide spread and indiscriminate use of antibacterial agents in hospitals had led to the progressive development of resistance to penicillin and many of the other antibiotic agents, by a large variety of important bacteria concentrated in the hospital environment. These virulent organisms have shown the potential to become pathogenic in patients weakened by disease, injury, metabolic conditions, surgery and other debilitating factors [10,11].

Hence the present study is carried out to determine incidence of surgical wound infection with the resistance pattern of the organisms which is helpful in selecting antimicrobial therapy for formulating infection control measures.

Material and Methods

The present study was conducted in the department of Microbiology peoples medical college and tertiary care hospital, Bhopal during the period from September 2013 to August 2014. 150 samples were collected from admitted patients of post operative wounds of various wards.

Inclusion Criteria

All post operative wounds of elective and emergency surgeries with sign of inflammation serous, sanguineous or purulent discharge, soaked dressings on gaping wound will be included in the study. All clean, clean- contaminated, contaminated, dirty type of wound will be included in the study (CDC Criteria).

Exclusion Criteria

1. Refusal to participate in study
2. Patient undergoing reoperation
3. Patient who were falling to come for a follow-up to 30 days since the day of operation.

Wound infection was diagnosed if any one of the following criteria were fulfilled:

a. Serous or non- purulent discharge from the wound,

b. Pus discharge form the wound

c. Serous or non- purulent discharge form the wound with signs of inflammation(oedema, redness, warmth, increased local temperature, fever ≥ 38°C, tenderness) [12].

Swabs were obtained from wounds and were processed without delay using standard microbiological methods.

Wound class was considered as clean, clean contaminated, contaminated and dirty as per center disease control classification. This classification is based on the extent of intra- operative contamination.

The data collected includes age and sex of patients, Diabetes, preoperative hospital stay, details of timing of antimicrobial prophylaxis, type of surgery (emergency and elective surgery), duration of surgery, presence and absence of drains, the wound classes.

According to the criteria of cutting and harding 13. wounds were considered infected and samples were collected from these infected wounds taking all aseptic precautions and then transported to the laboratory without delay.

Methodology

Swabs were collected form discharge of post operative wound. All the wound swabs were subjected to gram staining followed by inoculation in 5% sheep agar and Maconkey media. They were incubated aerobically at 37 degree Celsius for 24 hours and the colony characters were studied, Organisms were confirmed by following biochemical tests.

For gram positive cocci- catalase test, coagulase test, bile esculin test was done.

Lactose fermenting gram negative bacilli and non lactose fermenting bacilli were differentiated on maconkey agar (Non lactose fermenting) gram negative bacilli were tested by oxidase test. Both lactose fermenting and non lactose fermenting isolates were subjected to indole test, MR test, Urease test , Citrate test VP test, Nitrate test and (TSI) Sugar fermentation test, Motility was done by hanging drop method.

Antimicrobial susceptibility testing of all isolates was performed by Kirby baurs disc diffusion method as per CLSI guidelines [14]. Antibiotic discs were obtained from high media company. Turbidity of suspension was compared with 0.5 Macferlands standards [15].

Control strains used in study were-

- Escherichia coli ATCC 25922
- Staphylococcus aureus ATCC 25923
- Pseudomonas aeruginosa ATCC 27853

Results

- In the present study out of 1380 surgeries studied, the overall percentage of surgical wound infection was 10.8 % (**Table 1**)

Table 1: Showing percentage of the total infected cases

Total Surgeries	Total infected cases	Percentage (%)
1380	150	10.80

- Out of 150 infected cases 123 cases were culture positive while 23 were culture negative. ($Z \geq 1.96$) value was significant (**Table 2**)

Table 2: Showing culture positive cases and sterile cases

No. of infected cases	Growth	Sterile
150	127	23

- The percentage of infected wounds following emergency surgeries was 11.9 % followed by elective surgeries of 9.1%, but this rise was insignificant (P=0.110) (**Table 3**)

Table 3: Percentage of infection in type of surgeries

Type Surgery	Surgeries performed	No of infected cases	% Infection
Emergency	856	102	11.9
Elective	524	48	9.1
Total	**1380**	**150**	**100**

Maximum percentage of infection was seen in emergency surgeries 11.9% as compared to elective surgeries 9.1%. The difference was not statistically significant Chi square = 2.55, (P=0.110)

- The percentage of infected wound according to type of surgery was hight in dirty type (IV) surgeries i.e 29.06 % followed by contaminated type (III) surgeries with was 26 % & clean surgeries was 8% and clean contaminated surgeries was 7.05% ($p \leq 0.0001$) value was Significant (**Table 4**)

Table 4: Showing percentage of infection according to wound class

Wound class	Surgeries performed	No of infected cases	% infection
Clean	175	14	8
Clean Contaminated	964	68	7
Contaminated	69	18	26
Dirty	172	50	29.06
Total surgeries	**1380**	**150**	**100**

There was significant association between type of wound with infection Chi Square= 91.3 ($P \leq 0.0001$) According to study high percentage of infection was found in Dirty and Contaminated type of wound.

- The pathogens isolated were Staphylococcus aureus 22 (17.3%) Enterococcus 3 (2.36%), E coli 37 (29.1.%). pseudomonas aeruginosa 35 (27.5%, klebsiella pneumoniae 17 (13.3%), Acinetobacter species 5 (3.9%), Proteus mirabilis 5 (3.9) & citrobacter species 3 (2.3%) (**Table 7**)

Table 7: Frequency of various pathogens

Organisms	No of Isolates	%
E coli	37	29.1
Pseudomonas aeruginosa	35	27.5
Staphylococcus aureus	22	17.3
Klebsiella pneumonia	17	13.3
Acinetobacter spp	5	3.9
Proteus Mirabilis	5	3.9
Citobacter spp	3	2.3
Enterococcus	3	2.3
Total	**127**	**100**

-The percentage of infectivity was highest in incision & drainage (I & D) surgeries 95(28.4%) followed by ORIF i.e 87(25.2%) surgeries followedby debridement surgeries 67(23.8%) (**Table 9**)

Table 9: Percentage of infections in various surgeries

Total no. of Surgeries performed	Total no of surgeries infected	% infection
L.S.C.S. (313)	17(5.43)	5.43
Hystectomy (190)	9(4.7)	4.7
Hernioplasty (140)	10(7.1)	7.1
Cholecystectomy (140)	3	2.1
Laporatomy (82)	14	17.01
Appendictomy (140)	10	7.1
I & S (95)	27	28.42
Debridement (67)	16	23.8
ORIF (87)	22	25.2
Cytolithiotomy (30)	3	10
Amputation (45)	7	15.5
Craniotomy (22)	2	9.09
Other (29)	10	34.4
Total (1380)	**150**	

Highest percentage of infection was found in I & D surgeries (28.4%) , as compare to other surgeries. This difference was significant p?

- Staphylococcus aureus strains were 100% sensitive to Linezolid Vancomycin followed by cefoxitin 80% and Ceftazidime Claculanic acid 62% Staphylococcus aureus were 80% resistant to erythromycin and azithromycin folloed by ciprofloxacin 60% (**Table 13**)

Table 13: Antibiotic sensitivity pattern of gram positive isolates

Antimicrobials	Staphylococcus aureus N = 22		Enterococcus N= 3	
	No	%	No	%
Amoxyclav	13	60	2	66.6
Erythromycin	5	20	1	33.3
Ciprofloxacin	9	40	2	66.6
Ampicillin/ Sulbactum	11	50	2	66.6
Cefoxitin	17	80	1	33.3
Vancomycin	22	100	3	100
Linezolid	22	100	3	100
Clindamycin	13	60	1	33.3
Azithromycin	5	20	1	33.3
Cetazidime/ Clauvanic Acid	14	62	2	66.6

-Klebsiella pneumoniae were 98% sensitive to Colistin followed by Cefepime/ tazobactum 93% (**Table 14**)

Table 14: Showing antibiotic sensitivity pattern of gram negative isolates

Antibiotics	Pseudomonas Aeruginosa N =35		Escherichia coli N = 37		Klebsiella Pneumonae N 17		Acinetobacter Spp. N= 5		Citrobacter Spp. N = 3		Proteus mirabilis N = 5	
	No	%	No	%	No	%	No	%	No	%	No	%
Amikacin	11	32	26	73.5	9	56.3	1	20	3	100	2	60
Amoxyclav	18	53	7	20.6	1	7.8	2	40	3	100	5	100
Cefotaxime	6	19.1	8	23.5	3	23	4	80	1	33.3	5	100
Imipenam	30	87	36	98	12	75	4	80	3	100	5	100
Piperacillin Tazobactum	35	100	25	70	10	60	3	60	3	100	5	100
Cerftariaxone	25	72.2	11	30	3	16	1	20	3	100	1	20
Ceftazidime Claculanic Acid	27	77.6	29	79	15	90	4	80	3	100	5	100
Tobramycin	14	42	32	87	12	72	2	40	3	100	3	60
Colistin	21	60	27	65	16	98	2	40	2	66.6	3	60
Polymixin B	28	80	27	78	12	78	4	80	3	100	4	80
Ciprofloxacin	16	46	2	6	2	12	3	60	2	66.6	3	60
Cefipime/ Tazobactum	14	42	3	9	15	93	4	80	3	100	5	100

Discussion

Out of 1380 surgeries studied 150 were infected surgeries which included 1230 normal surgeries. Overall percentage of surgical wound infection in the present study was 10.8%. This was in agreeement with overall infection rate which ranged from 6.09 to 38.7%. 4.19, Infection rate in Indian hospital is much higher than other countries for instances USA it is 2.8 % & in European countries it is 2.6% & 9.5% higher infection rate in Indian hospital is due to poor setup of our hospitals and also due to lack of attention towards basic infection control measures. The overall infection rate varies from surgeon to surgeon, hospitals to hospitals, one procedure to another and from one patient to another patient and also depends on location of surgeries, bacterial load in tissues and integrity of host defenses [16].

Percentage of infective wounds in our studies is more in emergency surgeries 11.9% followed by elective surgeries was 9.1. This was similar to finding from study by patel sachin et al., satyanarayan v et al [17,18].

In our study by statistical analysis by chi square test the difference between emergency and elective surgeries regarding percentage of infection was not significant (P+ 0110).

Higher rate of infection in emergency surgeries may e because of insufficient pre operative preparation and more frequency of contaminated or dirty wound in emergency in surgeries.

In our study percentage of infected wound was higher in dirty type surgery i.e. 29.06% followed by contaminated surgery 26% followed by clean 8% and then clean contaminated i.e. 7%. These results were similar to study by anvikar et al and Olsen Marry et al. [18-22]. In our study on statistical analysis there was significant association between type of wound with infection (chi square = 91.3) ($p \leq 0.0001$).

In our study the percentages of infected cases with hospital stay ≥ 7 days was 25.3% followed by 4.05% in hospital stay ≤ 7 days [23].

In our study difference was statistical significant $p \leq 0.001$ by chi square test. Longer hospital stay leads to colonization with antimicrobial resistant organisms effects patient's susceptibility to infection by lowering host resistant or by providing increased after opportunity for ultimate bacterial colonization [12,17,20,23].

We found that out of total post operative infective cases 92 were males (61.3%) followed by 58 females (38.6%) . Thus in our study males were prone to SSI7,101. On comparison of percentage of infection on between males and females by statistical analysis by z rest (z1.96) was significant.

In our study maximum percentage of culture positive infected cases were in age group 0f 21 to 30 (26.7% followed by age group 30 to 40 (25.1%). [24].

In our study E. coli was the commonest organism isolated i.e. 37 (29.1) followed by pseudomonas aeruginosa 35 (27.5%) followed by staphylococcus aureus 22 (17.3). This was similar to the study by shittu et al and Patel sachin et al. [17,22].

Staphylococcus aureus were 100% sensitive to vancomycin and Llinezolid followed by Cefoxitin 80 % and clavulanic acid 62%, Staphylococcus aureus showed maximum resistance to Erythromycin 80% followed by Ciprofloxacin 60% [25]. Enterococcus is 100% sensitive to Vancomycin and Llinezolid and showed maximum resistance to Erythromycin followed by Cefoxitin.

Amongst the gram negative E coli was 98% sensitive to Imipenam followed by tobramycin 87% followed by ceftazidime / Clavulanic acid 79% then Polymyxin B 75% E coli strains showed maximum resistance to ciprofloxacin 94 % folowed by Amoxyclave 79.5%. [26].

Pseudomonas aeruginosa strains were 100% sensitive to Piperacillin tazobactum and were 80.9% resistant to Cefotaxime followed by Amikacin 68%.

Klebsiella pneumoniae were 98% sensitive to Colistin followed by Cefepime/ tazobactum 93%. Klebsiella pneumoniae were maximum resistant to Amoxy/ clave (7.8%)

The percentage of infectivity was hight in incision & drainage (I & D) surgeries 27(28.4%) followed by ORIF I.e 87 (25.2%) surgeries followed by debridement surgeries 67 (23.8). Highest percentage of infectivity was in I & D surgeries. This was similar to study by Mahesh CB, shiv Kumar et al. [4].

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Antimicrobial susceptibility patterns and risk factors profile of patients with septicaemic meliodosis: Single centre experience over a period of five years

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Abstract

Aims and Objective: In this paper, we review the antimicrobial susceptibility patterns and risk factors in patients with septicaemic meliodosis, seen over a period of five years - from 2010 to 2014. *Material and methods:* We have collected all blood culture of *B. pseudomallei* positive patients, between the periods January 2010 to December 2014 for the study. Identification of *B. pseudomalleifrom* positive blood cultures was done by cultural characteristics where all isolates were serologically confirmed using polyclonal antiserum raised in rabbits. Antimicrobial susceptibility testing was done using standardized protocols. Geometric mean MIC for each antibiotic for each year was calculated and reported. *Results:* A total of 54 patients were found to have *B. pseudomallei* septicaemia during the study period, 8 in 2010, 14 in 2011, 14 in 2012, 11 in 2013 and 7 in 2014. Of them, 47 (87%) were males, while the rest 7 (13%) were females. Ages of the patients ranged from 0 to 67 years (mean - 43.22±14.778).The organism was largely susceptible to ceftazidime, doxycycline, trimethoprim-sulfamethoxazole and carbapenems. We found only a single isolate of *B. pseudomallei* which showed resistance to meropenem and doxycycline. *Conclusion:* The study results re-assured that the organism is largely susceptible to routinely used antibiotics. The collation of MIC data over 5 years in the tertiary care institution was unable to reveal an evident MIC trend for ceftazidime, doxycycline, trimethoprim-sulfamethoxazole and carbapenems.

Keywords: Septicaemic Meliodosis; *B. Pseudomallei*; Antibiotic Sensitivity; Risk Factors.

Introduction

Melioidosis, caused by *Burkholderiapseudomallei*, has been on a steady rise, with an increasing number of cases being reported from the Indian subcontinent in the previous two decades [1]. The disease is endemic in Southeast Asia and northern Australia. A soil saprophyte, *B. pseudomallei* is a non-fermenting, oxidase positive gram negative bacilli. The organism is commonly transmitted via inoculation of contaminated soil through skin abrasions. Diabetes mellitus, chronic renal impairment pulmonary disease, thalassaemia,congestiveheartfailure, corticosteroid therapy, malignancy, immunosuppression and prolonged alcohol intake are known risk factors for melioidosis [2] The disease can have a wide spectrum of presentations ranging from localised, disseminated to septicemic.

The most serious form of the disease is an acute septicaemic illness which is associated with a high mortality rate. A correct diagnosis and prompt

initiation of appropriate treatment is crucial for patient survival. The organism is intrinsically resistant to a large number of antimicrobial such as penicillin, first and second-generation cephalosporins, aminogylosides and macrolides. The treatment of melidiosis thus consists of an initial intensive therapy with minimum of 10-14 days when ceftazidime or meropenem or imipenem is given, with or without sulfamethoxazole-trimethoprim. In the eradication phase, the backbone of therapy is sulfamethoxazole-trimethoprim for a minimum duration of 3 months, with or without doxycycline [3].

In this paper, we review the antimicrobial susceptibility patterns and risk factors in patients with septicaemicmeliodosis, seen overa period of five years- from 2010 to 2014. We aimed 1) to study the antimicrobial susceptibility patterns of bloodstream *B. pseudomallei* isolates. 2) to analyze the MIC trend of *B. pseudomallei* isolates over a period of five years (2010-2014). 3) to study the various risk factors associated with septicaemicmeliodosis.

Materials and methods

All blood culture *B. pseudomallei* positive patients, between the periods January 2010 to December 2014, were included in the study. Blood culture testing was performedin the BacTAlert automated system (bioMe´rieux). Identification of *Burkholderiapseudomallei* from positive blood cultures, was done by cultural characteristics and biochemical methods. All isolates were serologically confirmed using polyclonal antiserum raised in rabbitsin our hospital. Antimicrobial susceptibility testing was done by determining minimum inhibitory concentration (MIC) for ceftazidime, doxycycline, trimethoprim-sulfamethoxazole, meropenem (2010 and 2011 isolates) and imipenem (2012-2014 isolates), as per Clinical and Laboratory Standards Institute (CLSI M45) guidelines. Geometric mean MIC for each antibiotic for each year was calculated as it was a more sensitive indicator of MIC changes over the years.Clinical and demographic data was obtained from the medical records of the patient.

Results

A total of 54 patients were found to have *B. pseudomallei* septicaemia during the study period, 8 in 2010, 14 in 2011, 14 in 2012, 11 in 2013 and 7 in 2014. Of them, 47 (87%) were males, while the rest 7 (13%) were females. Ages of the patients ranged from 0 to 67 years (mean±SD 43.22±14.778). The table 1 gives an account of the various predisposing factors seen in the patients.

The table 2 depicts the number of isolates susceptible, intermediate or resistant to the various antimicrobials testedin each year. Trends of geometric mean MIC of B pseudomallei isolates over 5 years is presented in Table 3.

Discussion

There were 54 blood stream isolates of *B. pseudomallei*during the five year period from 2010 to 2014. Male preponderance was seen (87%), as has been reported in other studies [1,4] which is mostly due to the outdoor nature of the work. The mean age of the patients was 43 years. Diabetes was the commonest predisposing factor seen (55.5%). This is comparable to what has been reported in other studies (37-68%) [1,5]. Tuberculosis, hypertension, chronic kidney disease and transplantation were present in minority of patients.

In this study, the organism has been largely susceptible to ceftazidime, doxycycline, trimethoprim-sulfamethoxazole and carbapenems. Hence, the patients were treated with ceftazidime with or without meropenem in the intensive phase and sulfamethoxazole-trimethoprim with doxycycline in the continuation phase. Clinical

Table 1: Predisposing factors for melioidosis

Predisposing factor	N=54	Percentage (%)
Diabetes mellitus	30	55.5
Tuberculosis	8	14.8
Hypertension	3	5.5
Chronic kidney disease	2	3.7
Transplantation	1	1.9

Table 2: Antimicrobial susceptibility testing by MIC for B pseudomallei isolates from 2010-2014 (CLSI M45)

	Ceftazidime			Imipenem/Meropenem			Doxycycline			Cotrimoxazole		
	S ≤8.0	I 16	R ≥32.0	S ≤4.0	I 8	R ≥16.0	S ≤4.0	I 8	R ≥16.0	S ≤2/38	I -	R ≥4/76
2010	8	-	-	8	-	-	8	-	-	8	-	-
2011	14	-	-	13	-	1	13	-	1	14	-	-
2012	14	-	-	14	-	-	14	-	-	14	-	-
2013	11	-	-	11	-	-	11	-	-	11	-	-
2014	7	-	-	7	-	-	7	-	-	7	-	-

Table 3: Geometric mean MIC of B pseudomallei isolates over 5 years (2010 -2014)

Year	MIC (μg/ml) Ceftazidime	Imipenem	Meropenem	Doxycycline	Cotrimoxazole
2010	1.89	-	0.67	1.53	0.88
2011	2.33	-	0.61	1.91	0.35
2012	1.46	0.57	-	1.51	0.63
2013	1.82	0.50	-	1.69	0.99
2014	3.02	0.54	-	1.37	0.92

trial evidence supports the use of ceftazidime or a carbapenem antibiotic for initial parenteral therapy, which should be administered for at least 10-14 days. This is followed by a prolonged course of oral antimicrobial therapy with trimethoprim-sulfamethoxazole with or without doxycycline [6].

We found only a single isolate of B. pseudomalleiwhich showed resistance to meropenem and doxycycline. Resistance to these drugs is very uncommon [7,8]. Beheraet al from Andhra Pradesh, India, have reported a case of disseminated septicaemicmelioidosis with ceftazidime resistance [9]. Low rates of resistance have also been reported from Malaysia [10].

We attempted to study if any MIC trends could be made out over 5 years. However, there was no consistent trend for any antibiotic. The geometric mean MICs over five years did not show any evidence of an increasing trend.

Conclusion

Melioidosis is a largely an under-recognized infection and needs a high index of suspicion for diagnosis. Prompt initiation of treatment is essential, especially in the septicaemic form of the disease. It is re-assuring to find that the organism is largely susceptible to routinely used antibiotics. The collation of MIC data over 5 years in the tertiary care institution was unable to reveal an evident MIC trend for ceftazidime, doxycycline, trimethoprim-sulfamethoxazole and carbapenems.

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Molecular Detection of *Culicoides* Midges Responsible of BTV Transmission

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Abstract

Bluetongue (BT) is an infectious, non-contagious arthropod borne viral disease of wild and domestic ruminants especially sheep which inflict major losses on subsistence of sheep farmers in southern India. Affected sheep may have erosions and ulcerations on the mucous membranes, dyspnea, lameness and inflammation of the coronary band. The disease is caused by bluetongue virus (BTV), the type species of the genus Orbivirus and family Reoviridae. BTV is transmitted between their ruminant hosts by certain species of haematophagous insects such as Culicoides (biting midge). BT is OIE list 'A' multispecies disease. Till date more than 27 serotypes have been reported. The present study was carried out with the objectives to identify and characterize the Culicodes species available in our laboratory. The Culicoides DNA samples extracted using a non-destructive DNA extraction method were amplified using mitochondrial cytochrome oxidase I (COI) gene specific primers. An amplicon of 523 bp was obtained in amplified sample. The samples were further morphologically confirmed as culicoides oxystoma by stereo microscope. In this study, 25 Culicoides samples were processed for molecular detection by PCR. Out of these 20 samples were identified as C. Oxystoma. Morphological study by stereo microscope also confirmed these 20 samples as C. Oxystoma.

Keywords: Bluetongue Virus; Non-Destructive DNA Extraction; *Culicoides Oxystoma*; PCR.

Introduction

Bluetongue (BT) disease is caused by Bluetongue virus (BTV). BTV is type species of genus *Orbivirus* within *Reoviridae* family. BT may cause infection in domestic and wild ruminants, camelids and occasionally in carnivores (Attoui *et al.*, 2009; Meyer *et al.*, 2009). BT is transmitted by biting of certain species of *Culicoides* (biting midges) vectors. BT causes huge economic losses to live stock sector and farming community. Globally, more than twenty seven distinct BTV serotypes have been recognized so far (Hofmann *et al.*, 2008; Maan *et al.*, 2011; Jenckel *et al.*, 2015). Including recent isolation of BTV5 and BTV24 a total of 24 different BTV serotypes have been reported from India (Prasad *et al.*, 2009; Krishnajyothi *et al.*, 2016; Hemadri *et al.*, 2016).

The BT virion particle is non-enveloped, icosahedral in structure. It has complex architecture with inner core and outer capsid. The inner core and outer capsid have diameter of 75 nm and 90 nm respectively (Grimes *et al.*, 1998; Nason *et al.*, 2004). BTV genome is made up of 10 segments

of dsRNA. The BTV genome segments encode 7 structural (VP1 to VP7) and 5 non-structural (NS1, NS2, NS3/NS3A, NS4 and NS5) proteins (Firth, 2014). The inner core of virus is composed of major (VP3 and VP7) and minor (VP1, VP4 and VP6) proteins which is surrounded by outer capsid, composed of VP2 and VP5 proteins. The virus specific non-structural proteins are produced only in infected host cells. The NS1 and NS2 proteins form tubules and inclusion bodies respectively in infected host cells.

India has tropical and rainy climate, which favour breeding of *Culicoides* vectors. Globally, more than 1400 different *Culicoides* species have been reported. Out of these, 63 are identified from different geographical regions of India such as Assam and West Bengal (Halder *et al*., 2013), Tamil Nadu (Ilango, 2006), Maharashtra (Narladakar *et al.,* 1993), Andhra Pradesh (Reddy and Hafeez, 2008) and Karnataka (Archana *et al*., 2014). Very few species of *Culicoides* have been demonstrated as vectors for BTV transmission. BTV serotype 1 was isolated from *Culicoides oxystoma* vector in Gujarat state (Dadawala *et al*., 2012). Recently, BTV was also isolated from *Culicoides schultzei complex* from West Bengal state (Joardar *et al*., 2016). There is a critical need of study to analyze the vector-virus relationship. Several *Culicoides* species have been reported as putative BTV vector in India viz., *Culicoides actoni, Culicoides brevitarsis Kieffer, Culicoides dumdum, Culicoides fulvus, Culicoides imicola Kieffer, Culicoides oxystoma Kieffer* and *Culicoides peregrinus Kieffer* (Prasad *et al*., 2009). However, this implication is reported based on vector competence data collected and analyzed in other countries. Recently, *Culicoides oxystoma* and *Culicoides peregrinus* were reported as potent vector of BTV 16 and 23 in India (Ranjan et al., 2017a; 2017b). However, there is a scanty data available on vector competence of *Culicoides* to BTV in India.

Several varieties of ecological zones are found in the Indian subcontinent which harbors various *culicoides* species responsible for BTV transmission. However, the *Culicoides* species which transmit BTV in Indian livestock and wild-life are not fully characterized (Patel *et al*., 2007). Establishing a fundamental base is prerequisite for *culicoides* species identification in India for understanding BTV epidemiology correctly. Moreover, questions relating to *culicoides* populations phylogenetic and taxonomic relationships also exist within India with other global *culicoides* vector species. These questions may be answered through the morphological and sequence based molecular identification of *Culicoides* spp. from India. Thus keeping the above perspective in view, the present study was proposed for molecular and morphological detection of *culicoides* vector responsible for bluetongue virus infection.

Materials and Methods

Samples

A total of 25 *Culicoides* samples, available in our laboratory were used for molecular detection and morphological identification.

Culicoides *species identification*

The *Culicoides* species were detected using PCR and morphologically confirmed by stereo microscopy.

Extraction of DNA from ***Culicoides*** *samples*

The total *Culicoides* DNA was extracted using a non-destructive method standardised in lab and species identification was done through *cytochrome oxidase I* (*COI*) gene specific PCR.

Incubation of samples

Culicoides DNA extraction was done using non-destructive method standardised in laboratory. An aliquot of 200 µL of digest solution was taken in sterile and labelled 1.5 mL tubes. The *Culicoides* midges abdomen region were individually transferred to tube containing digest solution and incubated at 40^0C for 16 hours in a thermal-cycler (Quanta Biotech, USA). The *Culicoides* samples were removed from the tube and remaining mixture was further incubated at 70^0C for 15 minutes to inactivate the enzyme. Reaction tubes were removed from thermal-cycler and stored at 4^0C till further use.

Precipitation of DNA

In a 1.5 mL micro centrifuge tube, 20 µL Sodium Acetate, 600 µL ice cold 100% ethanol and 1µL glycogen were added sequentially. The DNA mixture stored at 4^0C was added to this tube. The resultant mixture was mixed and stored at -20^0C for 1 hours followed by centrifugation at 13,000 rpm (10,000Xg) at 4^0C from 30 minutes. Supernatant was discarded and resultant pellet was air dried and dissolved in 50 µL of elution buffer (10mM Tris HCL, pH 8.0) and used for PCR assay.

*Amplification of **Culicoides Cytochrome oxidase I** (COI) gene by PCR*

Culicoides spp. identification was done on the basis of mitochondrial COI gene sequences (Dallas *et al*., 2003). The PCR assay was conducted in a 20 µL volume containing 5X Phusion HF Buffer 4 µL, DMSO 0.6 µL, 10mm dNTPs mix. 0.4 µL, Phusion polymerase enzyme 0.2 µL, 0.2 µL of 15 µM of each forward (C1-N-2191-mod: 5′ CCCGGTAAAATTAAAATATAAACTTC 3′) and reverse (C1-J-1718-mod: 5′ GGAGGATTTGGAAATTGATTAGTTCC 3′) primer (Dallas *et al*., 2003), 2 µL of DNA template and 12.4 µL of nuclease free water. Thermal condition was set as initial denaturation at 98 °C for 2 minutes followed by 35 cycles of denaturation at 98^{0}C for 15 second, annealing at 56^{0}C for 20 second, elongation at 72^{0}C for 30 second. Final elongation was done at 72^{0}C for 10 minute.

Visualization of amplified PCR products by agarose gel electrophoresis

The amplified PCR products were analysed on 1.0% agarose gel containing 0.5 µg/mL of ethidium bromide. The gel was visualized under UV trans-illuminator (Biovis, USA).

Results and Discussion

*Molecular detection of Indian **Culicoides** species*

For confirmatory identification of various *Culicoides* species, molecular technique such as PCR based detection system is essential. DNA from a total number of 25 *Culicoides* samples was isolated using non-destructive method. Out of these, 20 were found PCR positive with C1-N-2191-mod and C1-J-1718-mod COI gene specific primers with the amplified region of 523 bp (Figure 1).

*Morphological identification of **culicoides** samples*

Further for the confirmation of species of *culicoides* vector morphological identification through wing pattern of *Culicoides* sp. was done under stereo microscope using standard illustration (Bellis, 2014). All the 20 samples showed morphological feature similar to *Culicoides oxystoma* of apical and basal pale markings in anal cell (bb), apical pale spot in cell m1 not reaching to margin of wing (bbb), and pale spots in anal cell (bbbb). Hence, all *culicoides* samples were found to be *culicoides oxystoma* (Figure 2).

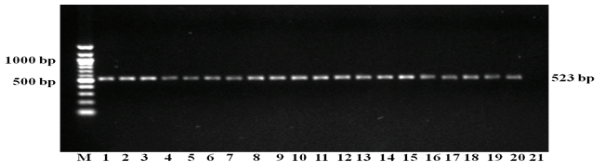

Fig. 1: Agarose gel electrophoresis of *Culicoides oxystoma* samples, showing 523 bp amplification of COI gene. Lane M: 100bp marker, Lanes 1-20: *Culicoides oxystoma* samples, Lane21: Nuclease free water negative control.

Discussion

BTV is transmitted by certain species of *Culicoides* vectors which is highly dependent on environmental changes (Wittmann *et al*., 2001). Along with vector species, environmental changes can influence the incidence and evolution of vector transmitted infectious diseases such as BT (Jimenez-Clavero, 2012). Recent study by several researchers have shown the changes in global pattern of BTV serotype distribution with introduction of exotic BTV strains and serotypes in India, Europe, Australia, Korea, North America, Middle east and South America (Shimshony,

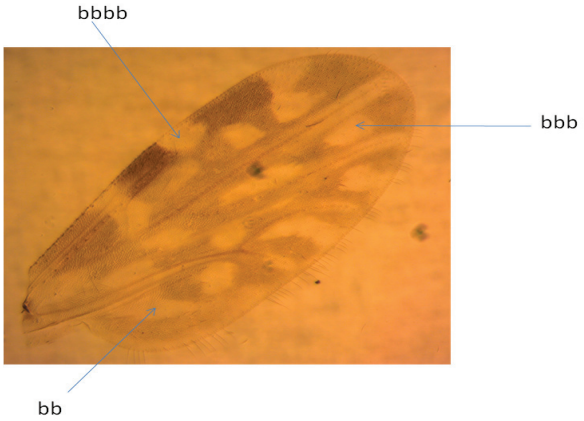

Fig. 2: Morphological identification of *Culicoides oxystoma* through wing pattern

2004; Brenner *et al*., 2010; Maan *et al*., 2012; Boyle *et al*., 2012; Maclachlan *et al*., 2013). This changed global pattern of BTV distribution can be linked to several factors such as climatic changes, increased travel and trade (Purse *et al*., 2005). As far as BTV transmission through *Culicoides* vector in India is concerned, very limited information is available. In India, first evidence of involvement of *Culicoides* sp. in BTV transmission was reported in Haryana state (Jain *et al*., 1988). Subsequently, *Culicoides oxystoma* was identified from animal farms where BTV sero-conversions were reported (Bhatnagar *et al*., 1997).

The tropical humid climate favors the *Culicoides* vector breeding. About 63 different *Culicoides* species have been morphologically identified from different regions of India (Reddy and Hafeez, 2008; Halder *et al*., 2013; Archana *et al*., 2014). Molecular identification of *Culicoides* species has also started in India (Minakshi, 2014; Ranjan et al., 2017a; Ranjan et al., 2017b). For species confirmation of *Culicoides* through molecular technique total DNA was extracted from abdomen region of *Culicoides sp*. using non-destructive method and allowed for COI gene based PCR (Dallas *et al*., 2003) followed by nucleic acid sequencing. The abdomen region of *Culicoides* vector was also used earlier by researcher for DNA extraction followed by species confirmation using COI gene based PCR and nucleic acid sequencing (Foxi *et al*., 2016). The morphological identification is considered as robust technique for putative BTV vector (*Culicoides* species) identification, with no misidentifications of *Culicoides* species (Harrup *et al*., 2016).

The earlier researcher had also carried out the COI gene sequence and DNA barcoding based species confirmation of some of the *Culicoides* species such as *Culicoides Imicola, Culicoides actoni, Culicoides brevitarsis, Culicoides oxystoma, Culicoides peregrinus, Culicoides brevitarsis, Culicoides huffi, Culicoides innoxius, Culicoides kepongensis, Culicoides mesghalii, Culicoides peliliouensis, Culicoides peregrinus* and *Culicoides similis* in Southern India (Harrup et al., 2016). Seven new *culicoides* species has been reported for the Tunisian Funna and out of them five were suspected/confirmed for either BTV or EHDV vector (Sghaier et al. 2017). The subgenera contain *Culicoides, Monoculicoides*. *Hoffmania, Haematomyidium* and *Avaritia* (the main vectors for the bluetongue viral disease) are monophylatic, whereas *Oecacta*, the subgenus is paraphyletic (Augot et al. 2017). Thus, mitochondrial COI gene based molecular identification along with morphological study of wing pattern can be used for *Culicoides* species confirmation.

Conclusion

DNA was extracted using non-destructive method from abdomen of all *culicoides* samples. The extracted samples were invariably amplified by polymerase chain reaction to amplify the desired size of COI gene. Out of the 25 samples, 20 were found positive and amplified the product size of 523 bp. Morphological identification were found similar to the *C. oxystoma*.

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Original Article

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Anti-Hepatitis B surface antigen level amongst vaccinated health care workers in a tertiary care hospital

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Abstract

Context: Hepatitis B virus infection is a global health problem. Centre for Disease Control and prevention recommends vaccination for all health care workers. Protection statuses of vaccinees need to be assessed by measuring anti- Hepatitis B surface antigen level in the serum. *Aims:* This study aims to detect the serum levels of anti-Hepatitis B surface antigen level among vaccinated health care workers of our Institution. *Settings and Design:* Cross-sectional study *Methods and Material:* This study included 93 heath care workers (surgeons, physicians, dentists, medical students, nurses, laboratory technicians, blood bank personnel and hospital attendants) of our tertiary care hospital for testing of anti-Hepatitis B surface antigen level; liver function tests were also done for the participants. *Statistical analysis:* Data analysis was done using statistical package for social sciences version 22. Percentages were calculated for categorical variables, Chi-square test was used for comparison of proportions. Logistic regression analysis was performed for studying the factors influencing response to vaccine. *Results:* In the present study, it was observed that about 9.7% were non-responders, 21.5% were poor responders and the rest (68.8%) were responders. *Conclusions:* All vaccinated individuals cannot be presumed to be fully protected. It is imperative to test the anti-Hepatitis B surface antigen level of the individual after completing the course to decide on appropriate measures to be taken.

Keywords: Hepatitis B Surface Antigen; Heath Care Workers; Responders; Vaccinees.

Introduction

Hepatitis B is a viral infection of liver transmitted through blood or body fluids. About one-third of world's population have been infected with Hepatitis B Virus (HBV) [1]. HBV is an important occupational hazard among health care workers (HCWs). Centre for Disease Control and Prevention (CDC) has recommended that all HCWs should be vaccinated against HBV. Protection statuses of

HBV vaccinees are determined by measuring anti-Hepatitis B surface (anti-HBs) antigen level in the serum. However, the immune response to HBV vaccine is variable and influenced by several factors. About 12-21% of HCWs are non-responsive to HBV vaccine. [2] Although HBV infection is a major health care issue, studies assessing the immune response among vaccinated HCWs in India are lacking. Therefore, this study was conducted to assess the protection status of HBV vaccinated HCWs by determining their serum level of anti-HBs antigen.

Materials and Methods

A cross-sectional study was conducted among 93 HCWs (surgeons, physicians, dentists, medical students, nurses, laboratory technicians, blood bank personnel and hospital attendants) in our tertiary care hospital for testing of anti-HBs antigen level; biochemical parameters used in the assessment of liver function were also included in the study. HCWs not vaccinated against HBV and with positive history of HBV infection were excluded. Informed consent was obtained from all the participants and Institutional Human Ethics Committee (IHEC) approval has been obtained before commencement of the study.

1. Five ml of venous blood was collected and Enzyme-linked immunosorbent assay (ELISA) was performed for detecting anti-HBs antigen level. All vaccinated health care personnel with anti-HBs level ≥ 100 m IU/ml were considered to have a full response; those with anti-HBs level between 10 and 100 m IU/ml were considered to have a poor response, while those with anti-HBs level < 10 m IU/ml were regarded as non-responders.
2. Liver function test (LFT) - total serum protein, serum albumin, total bilirubin, direct bilirubin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) levels were determined as per standard protocol.
3. Based on the grades of protection, the HCWs with poor response to the vaccine (anti-HBs antigen level between 10 and 100 m IU/ ml) were advised to receive a single booster vaccination and non-responders were advised to undergo tests to exclude current or past HBV infection, and given a repeat course of 3 vaccinations, followed by retesting after the second course of vaccination [3].
4. Data analysis was done using statistical package for social sciences (SPSS) version 22. Percentages were calculated for categorical variables, Chi-square test was used for comparison of proportions. Logistic regression analysis was performed for studying the factors influencing response to vaccine.

Observations & Results

A total of 93 HCWs who have been vaccinated for HBV were enrolled in the study. The demographic data of the 93 HCWs was summarized in table 1. Details regarding vaccination of HCWs were explained in table 2. The mean values of the anti-HBs antigen levels (m IU/ ml) of our study participants were shown in table 3. We evaluated the various potential factors that might have contributed to the non-responsiveness of HCWs to the vaccine and were summarised in table 4. The comparison of liver function tests of the HCWs with varying responses to the HBV vaccine was shown in table 5.

Table 1: Demographic details of study participants

Categories	Value
Age (Mean ± Standard deviation)	28.51 ± 7.02 (range, 19 to 62)
Sex:	
Male	55(59.1%)
Female	38(40.9%)
Speciality:	
Surgeons	23(24.7%)
Physicians	27(29.04%)
Medical students	11(11.8%)
Lab-technicians	2(2.2%)
Hospital attendant	2(2.2%)
Dentists	16(17.2%)
Nurses	9 (9.7%)
Blood bank personnel	3 (3.2%)
Body Mass Index	24.21 ± 3.85
Personal history:	
Smokers	7 (7.5%)
Chronic alcoholic	2 (2.2%)

Table 2: Vaccination details of study participants

Parameters	Value
No. of doses:	
One dose	8 (8.6%)
Two doses	16 (17.2%)
Three doses	51 (54.8%)
Three doses + booster	18 (19.4%)
Time interval between doses:	
1st and 2nd dose (n = 85) (Mean ± Standard deviation)	1.11± 0.98 months
2nd and 3rd dose (n = 69) (Mean ± Standard deviation)	6.00± 0.54 months
3rd and booster dose (n = 18) (Mean ± Standard deviation)	3.72± 1.9 years
No. of years since the first dose (Median, 25th and 75th percentiles)	6 (4 and 10)

Table 3: Mean values of anti-Hepatitis B surface antigen levels (m IU/ ml)

Category of responders	Mean ± Standard deviation (range)
Non-responders (n = 9)	1.21 ± 1.94 (0 to 6)
Poor responders (n = 20)	47.05 ± 27.00 (10 to 98)
Full responders (n = 64)	754.19 ± 342.32 (100 to 1000)

Table 4: Factors contributing to non-responsiveness to Hepatitis B vaccine

Risk factor	Non-responders n = 9 (%)	Responders n = 84 (%)	Relative risk	p value
Female sex	4 (44.4%)	34 (40.5%)	1.16 (0.33 to 4.03)	1.0000
> 25 yrs at primary vaccination	5 (55.6%)	16 (19.0%)	4.29 (1.26 to 14.54)	0.0255
>5 yrs after last dose	5 (55.5%)	38 (45.2%)	1.45 (0.42 to 5.07)	0.7281
Single dose	2 (22.2%)	6 (7.1%)	3.04 (0.75 to 12.24)	0.1717
Smokers	2 (22.2%)	5 (6.0%)	3.51 (0.89 to 13.81)	0.1357

Table 5: Comparison of the liver function tests of participants

Parameter	Mean ± Standard deviation			*p* value
	Non responder (n = 9)	Poor responder (n = 20)	Full responder (n = 64)	
Total protein (g/dl)	7.29 ± 0.62	7.51 ± 0.49	7.49 ± 0.45	0.4681
Albumin (g/ dl)	4.60 ± 0.31	4.74 ± 0.25	4.72 ± 0.33	0.5108
Globulin (g/ dl)	2.69 ± 0.63	2.77 ± 0.43	2.77 ± 0.46	0.8898
Total bilirubin (mg/ dl)	0.88 ± 0.68	0.60 ± 0.26	0.65 ± 0.34	0.1578
Direct bilirubin (mg/ dl)	0.37 ± 0.24	0.26 ± 0.09	0.27 ± 0.13	0.1037
AST* (units/ L)	29.89 ± 4.89	26.05 ± 7.25	29.36 ± 23.11	0.7919
ALT (units/ L)	21.56 ± 10.32	18.50 ± 10.08	24.70 ± 18.73	0.3373
Alkaline phosphatase (units/ L)	115.44 ± 39.96	110 ± 24.67	114.41 ± 29.83	0.8310

*AST-aspartate aminotransferases, ALT-alanine aminotransferases

Discussion

HBV vaccination is essential for all HCWs as they are at an increased risk of HBV infection. Although most HCWs receive the HBV vaccine as recommended by the CDC, many of them do not test their anti-HBs antigen level to know their protection status. We have assessed the immune response among 93 vaccinated HCWs of a tertiary care teaching hospital.

In our study, age of the participants ranged between 19 and 62 with the mean age of 29. Males were predominating in our study population. Among the participants, physicians participated in higher numbers (29%) followed by surgeons (25%)(table 1). Least number of participants was seen in the category of laboratory technicians and hospital attendants.

Enquiry regarding the vaccination details showed that only 19% of our participants had received complete vaccination including booster dose. Among the HCWs of our study, 51% had received three doses of HBV vaccine, but failed to take the booster. Incomplete vaccination (single

dose and two doses) was seen among 25.8% of our study subjects (table 2). In a similar study done by Pathak R et al in North India showed that only 38.8 % were completely vaccinated against HBV. Incomplete vaccination was seen in 21.4% and 40% of HCWs did not receive HBV vaccine at al [4]. Since our study aimed at studying the anti-HBs antibody level among HCWs, unvaccinated individuals were not included in this study.

In the present study, it was observed that about 9.7% were non-responders and 21.5% were poor responders (table 3). This emphasizes the fact that all vaccinated individuals cannot be presumed to be fully protected. It is imperative to test the anti-HBs antigen level of the individual after completing the course to decide on appropriate measures to be taken. Mean value of anti-HBs antigen level seen among full responders, poor responders and non-responders were 754, 47 and 1 m IU/ ml respectively. In similar studies done among HCWs in Pakistan and Sri Lanka, the percentage of non-responsiveness to HBV vaccine was 14% and 9.9% respectively [2,5].

The immune response to HBV vaccine is variable and can be influenced by several factors. We found that the individuals who received the first dose of HBV vaccine after 25 years of age had 4 times increased risk of being non-responsive to the HBV vaccine (table 4). Logistic regression analysis also showed that age > 25 years at primary vaccination is an independent risk factor associated with poor responsiveness to the HBV vaccine. Also inadequate vaccination (i.e., a single dose) was associated with about 3 times increased risk of non-responsiveness to HBV vaccine, but it was not found to be statistically significant as majority of the study participants have received three doses and only a few had received a single dose. Similar studies from other parts of the world have reported that age > 25 years and inadequate vaccination schedule were associated with increased risk of non-responsiveness to HBV vaccine [6,7]. Female sex and more than 5 years post vaccination were not suggestive of reasons for non-responsiveness. Smokers had 3.5 times increased risk of being non-responsive to HBV vaccine. Statistical significance between smoking and poor response to HBV vaccine was not established as only a few of the recruited participants were smokers. In a study assessing the responsiveness of the public safety personnel to HBV vaccine, smokers were observed to show a poor response to the vaccine [8]. Elderly individuals, patients with chronic diseases and immune defects are the common non-responders [9]. Age more than 40 years, male gender, obesity, smoking and presence of other illness are possible risk factors for non-responsiveness to HBV vaccine [10].

All the HCWs included in the study had normal serum total protein and albumin levels (i.e., none of them had hypoproteinemia, which might hinder the response to HBV vaccine) (table 5). Also, no association could be established between the routine biochemical parameters used in the assessment of liver function and serology.

Conclusion

About one-third of the HBV vaccinees were either non-responders or poor responders. It is therefore imperative to test the response to vaccine after completing the course, so that appropriate measures can be taken. Age > 25 years at primary vaccination is an independent risk factor associated with poor responsiveness to the HBV vaccine.

Acknowledgement

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Conflicts of interest: None to declare

Key Messages

About 10% and 20% of Hepatitis B vaccinated individuals were non responders and poor responders respectively, who need repeat vaccination of full course or single shot based on their anti-HBs antigen level.

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Original Article

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Dermatophytoses: Prevalence, Isolation and Identification at a Tertiary Care Hospital in Hyderabad Karnataka Region

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Abstract

Introduction: Dermatophytosis is superficial fungal infection caused by dermatophytes. North Karnataka is well known for its hot and humid conditions making its population at risk for many fungal infections. Hence this study was undertaken to know the prevalence of dermatophytoses in our tertiary care hospital at Gulbarga and to isolate and identify the most common dermatophyte causing tinea infection in the said population. *Materials and methods:* This is a nine months study where a total of 195 samples from patients who were clinically suspected to have dermatophytoses were collected. Direct examination for fungal elements was done by using 10% KOH for skin and hair samples and 20% KOH for nail samples. Samples were cultured on Sabouraud's dextrose agar (SDA) with gentamicin and cycloheximide (SDA with actidione). Samples were inoculated and incubated at 37^{0}C and another set at 25^{0}C in BOD incubator. Identification of fungal growth was done by macroscopic examination of colony morphology, pigment production and microscopic examination by lactophenol cotton blue preparation. *Results:* In this study, out of 195 clinically suspected cases, 142 (72.82%) samples were positive in direct examination and 66 (33.84%) cases were culture positive. Most number of clinical cases was observed between age groups of 0–20 (57.43%) years and in males (62.05%). The most common clinical presentation of tinea infection in the patients coming to our hospital was T.corporis (35.38%). Most of the culture positive samples were from the age group of 0-10 years and most common clinical presentation among the culture positive samples was T.corporis. Out of 66 culture positive cases, the most common dermatophyte species isolated was Trichophyton rubrum (21, 31.81%), followed by Trichophyton mentagrophytes (17, 25.75%).*Conclusion:* Most common dermatophyte causing infection was Trichophyton and most common species causing infection in patients coming to our hospital was Trichophyton rubrum.

Keywords: Dermatophytes; Trichophyton; Trichophyton Rubrum; Trichophyton Mentagrophytes; Tinea Corporis.

Introduction

Dermatophytosis is superficial fungal infection caused by dermatophytes, a group of fungi that are capable of growing by invading keratin of skin, hair, and nails and include three genera Trichophyton, Microsporum, and Epidermophyton. Invasion of keratin is aided by keratinases found exclusively

in dermatophytes [1]. Infection is acquired by the deposition of viable arthrospores or hyphae on the skin surface of the predisposed individual. Arthroconidia adhere to the keratinized tissue and once established, the spores germinate and penetrate the stratum corneum causing infection.

Depending on their natural habitat, dermatophytes are classified as anthropophilic, zoophilic and geophilic species [2]. Anthropophilic species are derived from human hosts, zoophilic species are natural parasites of animals and geophilic species occur naturally in soil. The severity and chronicity of infection varies depending on the source, with anthropophilic species causing mild but chronic lesions, where as zoophilic species causing severe inflammatory lesions but readily curable [2].

Overcrowding, poor hygiene, low standards of living and high humidity contribute to the increased prevalence of dermatophytic infections [3]. Our tertiary care hospital caters to population from especially low socioeconomic status in Gulbarga. Moreover, Gulbarga district, situated in North Karnataka is well known for its hot and humid conditions making its population at risk for many fungal infections. Hence this study was undertaken to know the prevalence of dermatophytoses in our tertiary care hospital at Gulbarga and to isolate and identify the most common dermatophyte causing tinea infection in the said population.

Materials and Methods

This is a nine months study where a total of 195 samples from patients who came to Dermatology OPD of our hospital and were clinically suspected to have dermatophytoses were collected. A detailed history about age, gender, occupation, social status, duration of complaint and significant past history were taken.

After cleaning the lesions with 70% alcohol, scales were collected from erythematous growing margins of the lesion with a sterile blunt scalpel. Hairs were plucked with sterile forceps. Scrapings from the infected nail bed and from the undersurface of the nail as proximal to the cuticle were collected with a no.15 scalpel blade. Samples were collected in black sterilized Whartman paper and transported to the microbiology laboratory [3,4].

Direct examination for fungal elements was done by using 10% KOH for skin and hair samples and 20% KOH for nail samples. Samples were cultured on Sabouraud's dextrose agar (SDA) with gentamicin and cycloheximide (SDA with actidione). Samples were inoculated in two sets of culture media. One set was incubated at 37^0C and another set at 25^0C in BOD incubator. Cultures were examined twice weekly for the appearance of growth. Identification of fungal growth was done by macroscopic examination of colony morphology, pigment production and microscopic examination by lactophenol cotton blue preparation. Urease test was performed to differentiate Trichophyton species [3,4].

Results

In this study, out of 195 clinically suspected cases, 142 (72.82%) samples were positive in direct examination and 66 (33.84%) cases were culture positive. Most number of clinical cases was observed between age groups of 0–20 (57.43%) years and in males (62.05%). The age and gender distribution of collected samples are displayed in Table 1 and Figure 1 respectively

The most common clinical presentation of tinea infection in the patients coming to our hospital was T.corporis (35.38%), followed by T.capitis (21.53%), T.faciei (11.79%), T.cruris (7.69%), Onychomycosis (4.61%), T.pedis (4.61%), T.incognito (3.58%) and T.mannum (3.07%). Nine patients (4.61%) presented with extensive dermatophytoses with both T.corporis as well as T.cruris types and six patients

Table 1: Age distribution of samples in study

Age group	Number of samples	Percentage
0-10 years	61	31.28%
11-20 years	51	26.15%
21-30 years	20	10.25%
31-40 years	24	12.30%
41-50 years	9	4.61%
51-60 years	9	4.61%
Above 60 years	21	10.76%

(3.07%) presented with both T.pedis and T.mannum. The clinical presentations of dermatophytoses in our study are demonstrated in Table 2.

Sixty six samples were culture positive, among which 42 (63.63%) samples were from male patients and the remaining 24 (36.36%) samples were from female patients. Most of the culture positive samples were from the age group of 0-10 years (Fig 2) and most common clinical presentation among the culture positive samples was T.corporis in our study. (Table 3)

Out of 66 culture positive cases, the most common dermatophyte species isolated was Trichophyton rubrum (21, 31.81%), followed by Trichophyton mentagrophytes (17, 25.75%), Trichophyton schoenleinii (16, 24.24%), Trichophyton tonsurans (7, 10.60%), Epidermophyton floccosum (3, 4.54%) and Microsporum gypseum (2, 3.03%). (Table 4)

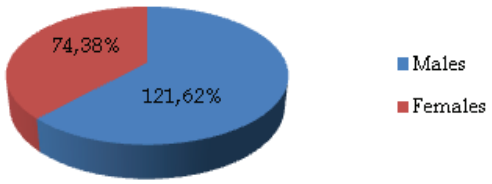

Fig. 1: Gender distribution of samples in study

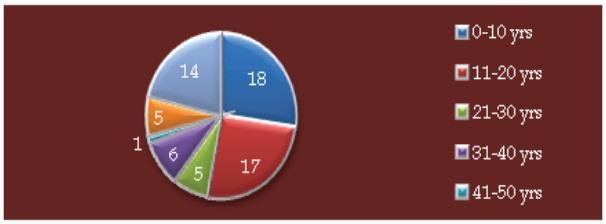

Fig. 2: Age distribution of culture positive samples

Table 2: Clinical presentations of dermatophytoses

Dermatophyte infection	Number of patients	Percentage
T.corporis	69	35.38%
T.capitis	42	21.53%
T.faciei	23	11.79%
T.cruris	15	7.69%
T.corporis/T.cruris	9	4.61%
Onychomycosis	9	4.61%
T.pedis	9	4.61%
T.incognito	7	3.58%
T.pedis/T.mannum	6	3.07%
T.mannum	6	3.07%

Table 3: Clinical presentations of culture positive cases

Dermatophyte infection	Number of cases	Percentage
T.corporis	23	34.84%
T.capitis	21	31.81%
T.cruris	8	12.12%
T.corporis/T.cruris	8	12.12%
Onychomycosis	4	6.06%
T.incognito	1	1.51%
T.faciei	1	1.51%

Table 4: Dermatophyte species isolated

Dermatophyte species	Number of isolates	Percentage
Trichophyton rubrum	21	31.81%
Trichophyton mentagrophytes	17	25.75%
Trichophyton schoenleinii	16	24.24%
Trichophyton tonsurans	7	10.60%
Epidermophyton floccosum	3	4.54%
Microsporum gypseum	2	3.03%

Discussion

In this study, highest incidence of dermatophytosis was observed in the age group of 0-20 years and in males. Similar findings were found in studies by Bindu et al and Huda et al [8,9]. Males are involved in increased physical and outdoor activities associated with exposure to hot and humid conditions causing increased sweating favoring the growth of dermatophytes [5,67]. The lower incidence in females could be due to apprehension to report to hospital because of conservative practices in India [10]. The incidence of dermatophytic infections in our study was observed in the low socioeconomic group of people and rural background similar to studies done in our country [5,6,7,10]. This is because of unhygienic living conditions, overcrowding,

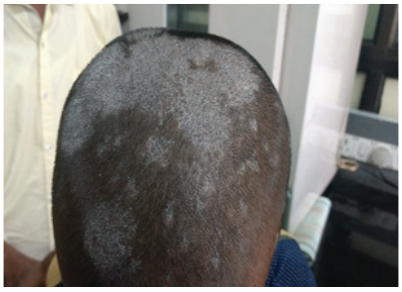

Image 1: Tinea capitis in a 8 year old boy

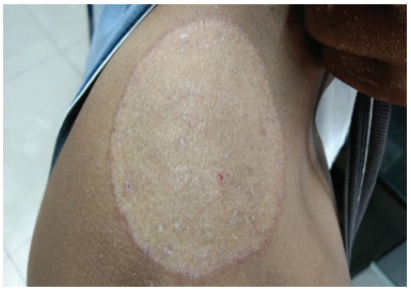

Image 2: Tinea corporis in a 28 year old man

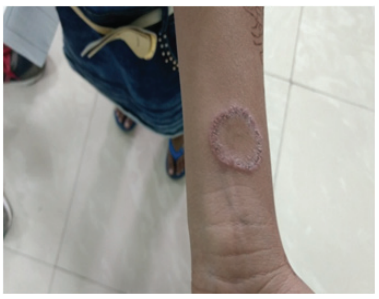

Image 3: Tinea corporis in a 6 year old girl

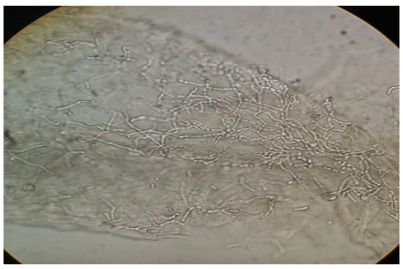

Image 4: KOH mount showing arthrospores

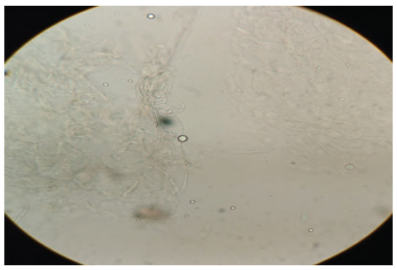

Image 5: KOH mount showing fungal elements in nail sample

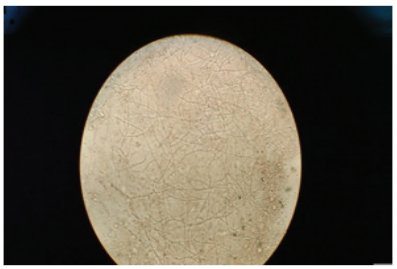

Image 6: KOH mount showing fungal elements in scalp scrapings

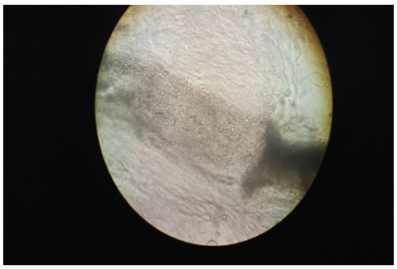

Image 7: KOH mount showing endothrix infection of hair

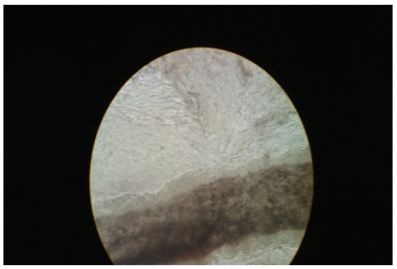

Image 8: KOH mount showing ectothrix infection of hair

Image 9: SDA slant showing growth of Trichophyton rubrum

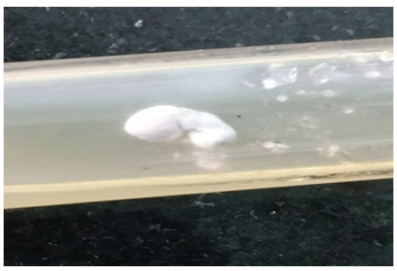

Image 10: SDA slant showing growth of Trichophyton mentagrophytes

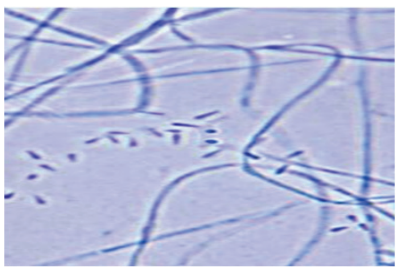

Image 11: LPCB mount showing Trichophyton rubrum

Image 12: LPCB mount showing spiral hyphae in Trichophyton mentagrophytes

illiteracy and poor nutrition among them. Increased humid weather and dusty environment of our district also predisposes the population for dermatophyte infections.

Tinea corporis was the most common clinical presentation of dermatophytosis in the present study which correlates with several studies done in India [6,7,8,9,10]. Most of the culture positive samples were from the age group of 0-20 years and most common clinical presentation among the culture positive samples was T.corporis in our study followed by T.capitis. This could be because the population coming to our hospital is predominantly Muslim population which is again predisposed to dermatophyte infections because of their cultural norms. The children aged 5-15 years are educated at religious schools (Madarasas) where they wear round caps causing increased sweating and moisture in the scalp region. These findings correlate with findings of Bindu et al and Noronha et al where higher incidence of T capitis was found in 0-10 years age group [8,10,11].

In this study, out of 195 clinically suspected cases, 142 (72.82%) samples were positive in direct examination and 66 (33.84%) cases were culture positive. This could be because of non viable fungal elements which fail to grow on artificial culture media. There was no KOH negative sample which showed growth on culture in our study. These figures are comparable to the KOH and culture findings of study done by Noronha et al [10].

Sixty six samples were culture positive, among which 42 (63.63%) samples were from male patients and the remaining 24 (36.36%) samples were from female patients. Most of the culture positive samples were from the age group of 0-10 years and most common clinical presentation among the culture positive samples was T.corporis in our study. This is similar to the findings of Siddappa et al. [12].

Out of 66 culture positive cases, the most common dermatophyte species isolated was Trichophyton rubrum (21, 31.81%), followed by Trichophyton mentagrophytes (17, 25.75%) in this study. This finding correlates with study of Singh et al and Bindu et al but is in contrast with Noronha et al where Trichophyton mentagrophytes was the predominant isolate [7,8,10]. This study shows Trichophyton mentagrophytes as the second most common isolate and the higher isolation rate may be due to changing trends in the prevalence of dermatophyte species in this part of Karnataka [10].

Preventive measures such as maintenance of personal hygiene, avoidance of tight and restrictive clothing and early diagnosis and treatment of clinically suspicious cases plays a major role in control of these infections in tropical countries.

Conclusion

Most common dermatophyte causing infection was Trichophyton and most common species causing infection in patients coming to our hospital was Trichophyton rubrum. This study shows Trichophyton mentagrophytes as the second most common isolate and the increased isolation rate may be due to changing trends in the prevalence of dermatophyte species in this part of Karnataka. Diagnosis of dermatophytic infections requires precise clinical examination supported by appropriate laboratory diagnostic aids. Early diagnosis and prevention of predisposing factors play a major role in control of dermatophyte infection.

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Antimicrobial susceptibility pattern and frequency of Acinetobacter species in different clinical specimens in a diagnostic centre of Madhya Pradesh

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Abstract

Introduction: Acinetobacter species are capable of causing infections such as pneumonia, sepsis in wounds, urinary infections, septicemia, endocarditis and meningitis. Apart from hospital acquired infections there has been a rising trend in community acquired infections with Acinetobacter species. It's intrinsic resistance and multi - drug resistance is posing a major health challenge. Acinetobacter species are becoming increasingly resistant to commonly used antibiotics such as aminoglycosides, fluoroquinolones, broad spectrum B-lactam antibiotics, cephalosporins and carbapenems.11 The aim of this study was to analyse the frequency of isolation of Acinetobacter species in various clinical specimens coming for culture in Microbiology department and also to assess antibiotic sensitivity pattern of the isolates. *Materials and Methods:* This prospective study was conducted between January to April 2018 in the Microbiology department of our centre. 2015 clinical specimens from both sexes and all ages like urine, pus, blood, different body fluids like pleural, ascitic, synovial, sputum and endotracheal tube etc were included in the study *Results:* Significant growth of Acinetobacter was observed in 10 (1.37%) cases .Acinetobacter was isolated from 3 Pus samples, 4 ET secretion samples, and one each from Urine, Throat swab and Body Fluid samples. The isolates were sensitive to only Colistin and Tigecycline with intermediate resistance to Ciprofloxacin and Cefepime and resistant to all the other antibiotics in all clinical specimens. *Conclusion:* Treatment of infections caused by Acinetobacter species is becoming increasingly difficult due to emergence of multidrug and pan drug resistant strains of Acinetobacter. The rampant and injudicious use of antibiotics is a major cause of this emerging drug resistance. A stringent hospital antibiotic policy and infection control policy should be implemented

Keywords: Acinetobacter Species; ET Secretions; Pan Drug Resistance.

Introduction

Bacteria of genus Acinetobacter are found everywhere. They are free living bacteria, saprophytic in nature and can be grown from soil, water, food and sewage [1].

Bacteria of Acinetobacter species are aerobic Gram negative coccobacilli which are glucose, non-

fermenters, oxidase negative and catalase positive and are opportunistic pathogens and are now-a-days an important source of nosocomial infection all around the world. They are commensals of skin and respiratory tract. Acinetobacter bauminii is the most frequently isolated pathogen [2]. The most important cause of its emergence in hospital acquired infections is it's capacity to survive in low humidity as well as developing resistance to antibiotics [3].

Long illnesses with hospitalization in ICUs and invasive procedures like catheterisation and ventilators can help in colonisation of these bacilli and can increase the risk of infection when immunity is low [4,5]. Acinetobacter species are capable of causing infections such as pneumonia, sepsis in wounds, urinary infections, septicemia, endocarditis and meningitis [6]. Apart from hospital acquired infections there has been a rising trend in community acquired infections with Acinetobacter species [7]. It's intrinsic resistance and multi - drug resistance is posing a major health challenge [8]. Throat, respiratory tract and digestive tract colonisation by Acinetobacter species have been documented in earlier outbreaks [9,10].

Acinetobacter species are becoming increasingly resistant to commonly used antibiotics such as aminoglycosides, fluoroquinolones, broad spectrum B-lactam antibiotics, cephalosporins and carbapenems [11].

The aim of this study was to analyse the frequency of isolation of Acinetobacter species in various clinical specimens coming for culture in Microbiology department of Sampurna Sodani Diagnostic Clinic and also to assess antibiotic sensitivity pattern of the isolates.

Materials and Methods

This prospective study was conducted between January to April 2018 in the Microbiology department of our centre. Clinical specimens from both sexes and all ages.

Like urine, pus, blood, different body fluids like pleural, ascitic, synovial, sputum and endotracheal tube etc were included in the study. A total of 2015 specimens were included in the study. Blood culture bottles were incubated in BacTalert (Biomerieux). Rest of the samples were cultured on Blood Agar and Mac-Conkey Agar and incubated at 37^0C for 24 hours. After 24 hours, Gram's staining was done from isolated colonies. Gram negative coccobacilli were identified as Acitenobacter by Vitek II (Biomerieux). Antibiotic susceptibility testing was done on Vitek II (Biomerieux) according to CLSI guidelines.

The patients were divided into 0-20, 21-40, 41-60, 61-80 and >80 years of age group in the both sexes.

Results

A total of 2015 specimens of all ages and sexes were included in the study out of which 917 were from males (45.5%) and 1098 (54.5%) were from females. The M:F ratio was 0.83:1 (Table 1). Out of 2015 specimens, 997 (49.4%) were urine cultures followed by 274 (13.5%) Pus swabs, Sputum 244 (12.1%), Blood cultures 178 (8.83%), Vaginal swabs 122 (6%) (Table 2).

Significant growth of Acinetobacter was observed in 10 (1.37%) cases and no growth was observed in 1288 (63.9%) patients (Table 2). Acinetobacter was isolated from 3 Pus samples, 4 ET secretion samples, and one each from Urine, Throat swab and Body Fluid samples. In 727 (36.1%) patients, bacteria other than Acinetobacter were grown. Maximum patients were between 21-40 years of age (33.8%), followed by 0-20 years (25.1%), 20.9% in 41-60 years, 17.7% in 61-80 years and least (2.33%) in patients above 80 years of age.

The isolates were sensitive to only Colistin and Tigecycline with intermediate resistance to Ciprofloxacin and Cefepime and resistant to all the other antibiotics in all clinical specimens. Table 3 shows the antimicrobial sensitivity pattern and MIC value of Acinetobacter species.

Table 1: Demographic Data of patients

S. No.	Age (years)	Male	%	Female	%	Total	%
1	**0 - 20**	266	29.0%	240	21.9%	506	25.1%
2	**21 - 40**	238	26.0%	444	40.4%	682	33.8%
3	**41 - 60**	173	18.9%	250	22.8%	423	21.0%
4	**61 - 80**	216	23.6%	141	12.8%	357	17.7%
5	**>80**	24	2.62%	23	2.1%	47	2.3%
Total		**917**	45.5%	**1098**	54.5%	**2015**	

Discussion

The bacteria of the genus Acinetobacter are lactose non fermenters, next only to Pseudomonas in the frequency of isolation from clinical specimens specially from hospital acquired infection [12].

Prolonged duration of hospital stay, immunocompromised status of patients, invasive diagnostic procedures and injudicious and rampant use of broad spectrum antibiotics has been a major trigger in colonisation and isolation of Acinetobacter species from various clinical specimens. This has also led to emergence of resistant strains of Acinetobacter species to commonly used antibiotics. Although these species are less virulent, but antibiotic resistance is rapidly acquired by these micro-organisms [13].

In our study, the maximum cases of Acinetobacter isolation was from ET secretions and were sensitive only to Tigecycline and Colistin while intermediate sensitive to Ciprofloxacin. All the 10 cases were resistant to Ampicillin, Amoxycillin/ Clavulinic acid, Piperacillin/ Tazobactum, Cefuroxime, Ceftriaxone, Cefepime, Imipenem, Meropenem, Gentamicin, Nalidixic Acid and Trimethoprim/ Sulfamethoxazole.

Hatice et al. in their study isolated 70% Acinetobacter species from tracheal aspirates, one from blood and wound infection (3.3%) and 6.6% from urine specimens which correlates with our

Table 2:

Sr No	Specimen	Overall Total Patients	Overall % of Total Patients	No Growth		Acinetobacter	
				Total Patients	%	Total Patients	%
1	Urine	997	49.40%	746	74.82%	1	0.10%
2	Blood	178	8.83%	103	57.87%	0	0.00%
3	Pus	274	13.50%	111	40.51%	3	1.09%
4	Sputum	244	12.10%	110	45.08%	0	0.00%
5	Stool	37	1.83%	33	89.19%	0	0.00%
6	Vaginal Swab	122	6.05%	93	76.23%	0	0.00%
7	Throat Swab	93	4.61%	47	50.54%	1	1.08%
8	CSF	15	0.74%	14	93.33%	0	0.00%
9	ET Secretion	11	0.54%	0	0.00%	4	36.36%
10	Body Fluid	29	1.43%	23	79.31%	1	3.45%
11	Semen	12	0.59%	8	66.67%	0	0.00%
12	Breast Abscess	3	0.14%	0	0.00%	0	0.00%
Total		**2015**		**1288**	**63.90%**	**10**	**1.37%**

Table 3: Sensitivity Pattern

Antimicrobial	MIC	Interpretation
Ampicillin	>=32	Resistant
Amoxicillin/ Clavulanic Acid	>=32	Resistant
Piperacillin/ Tazobactam	>=128	Resistant
Cefuroxime	>=64	Resistant
Cefuroxime Axetil	>=64	Resistant
Ceftriaxone	>=64	Resistant
Cefoperazone/ Sulbactam	>=64	Resistant
Cefepime	>=64	Resistant
Imipenem	>=16	Resistant
Meropenem	>=16	Resistant
Gentamicin	>=16	Resistant
Nalidixic Acid	>=32	Resistant
Ciprofloxacin	>=4	Resistant
Tigecycline	2	Sensitive
Nitrofuratoin	>=512	Resistant
Colistin	<=0.5	Sensitive
Trimethoprim/ Sulfamethoxazole	>=320	Resistant

study [14]. However, the isolates were sensitive to Netilmicin, Sulbactam, Amikacin and Meropenem also. While in our study, all antibiotics were resistant except Tigecycline and Colistin.

Muktikesh Dash et al. in their study of 8749 clinical specimens observed sterile specimens in 47.5% cases and Acinetobacter was grown in 3% cases [15]. They found 54.7% isolates to be pan drug resistant. Like our study, all the isolates were 100% sensitive to Colistin.

Rit et al in their study observed growth of Acinetobacter specimen in 3% specimens [16].

Mostofi et al and Joshi et al observed higher prevalence of Acinetobacter in their studies [17,18].

Vijayan et al in their study isolated Acinetobacter species from 122 cases out of 7182 clinical specimens, out of which 17.27% cases were pandrug resistant [19]. In their study, least resistance was seen to Piperacillin-Tazobactum and Imipenem.

The findings of pan drug resistance in our study show the emerging resistance to all antibiotics in Acinetobacter species. Hence, strong and effective infection control measures and judicious use of antibiotics is mandatory for prevention and effective treatment of infections with Acinetobacter species.

Conclusion

Treatment of infections caused by Acinetobacter species is becoming increasingly difficult due to emergence of multidrug and pan drug resistant strains of Acinetobacter. The rampant and injudicious use of antibiotics is a major cause of this emerging drug resistance. A stringent hospital antibiotic policy and infection control policy should be implemented.

Conflict of interest: none

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Study of bacteriology and antibiogram of diabetic foot infections

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Abstract

Inroduction: Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Diabetics exhibit a great frequency and severity of infection. A diabetic with foot complication spends 32.3% of the total income compared to 9.3% by the diabetic without foot complication *Materials & Methods:* . The sample taken was pus and exudates from the base of the ulcer after cleaning the necrotic tissue with a saline gauge. Direct Microscopy, Culture, Biochemical reactions & antibiotic sensitivity, *Results:* The male to female ratio was 2.03 in our study, The ulcers occurred over a wide range of age from 16 years to 78 years with a mean age of 47 years.64 out of 100 cases were between the ages of 45-65 years. There was only one case below 25 years of age and 3 cases above 75 years, Monomicrobial infections are more in grades–I &II and polymicrobial infections are more in grades– III & IV. *Conclusion:* The present study was conducted to know the bacteriology and antibiogram of diabetic foot infections in and aroundMangode, Palakkaddistrict Pseudomonas aeruginosa was the most common organism isolated and constitutes 32.43% of the total isolates Out of the 15 Pseudomonal isolates that were subjected to Screening of Amp – C β lactomase by diantagonism test only 4 isolates showed blunting of Cefotaxime zone of inhibition adjacent and to Cefoxitin and were considered screen positive

Keywords: Diabetic Foot; Amp – C; β Lactamase.

Introduction

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. It was once regarded as a single disease entity but it is now seen as a heterogeneous group of disease characterized by a state of chronic hyperglycemia resulting from a diversity of etiologies, environmental and genetic factors acting jointly [19].

Diabetics exhibit a great frequency and severity of infection. Out of all the infections, the foot infections are the most common for prolonged hospital stay amongst the diabetics. Diabetic foot is characterized by a classical triad of neuropathy, ischemia and infection. The foot infections are

a serious medical problem requiring prompt attention, appropriate diagnostic evaluation and proper therapeutic strategies [19].

Infection may be superficial or deep. Superficial infections are usually monomicrobial while deep infections are polymicrobial. A diabetic with foot complication spends 32.3% of the total income compared to 9.3% by the diabetic without foot complication [5].

The bacteriology and antibiotic sensitivity pattern of various pathogens in diabetic foot infections has been undergoing changes over the period of years. There is a considerable difference in the bacteriology of diabetic foot world wide in the different geographical areas [32].

Hence, the present study was conducted to establish the bacteriology and antibiotic susceptibility of diabetic foot infections, and an attempt was also made to know the resistance pattern of Pseudomonas aeruginosa which is noted for its multidrug resistance.

Materials & Methods

The material for the present study was collected from patients admitted in the departments of Surgery, Septic ward in Kerala Medical college Hospital

Study group

Diabetic patients presenting with ulcer on the foot belonging to Wagner's grades 1, 2, 3 & 4 were included.

Sample Collection

The samples were collected on the first day of admission. The sample taken was pus and exudate from the base of the ulcer after cleaning the necrotic tissue with a saline gauge.

The material was collected by swabbing the base of the ulcer with two sterile swabs, one for direct smear and the other for inoculating into liquid and solid media.

The specimens were processed by :

1. Direct microscopy
   a) Gram's Staining
   b) Hanging drop preparation and
   c) Capsular staining.
2. Culture
3. Biochemical reactions and sugar fermentation tests
4. Antibiotic susceptibility of the isolates was performed by Kirby – Bauer disc diffusion method

Results

In the present study 100 samples of pus and exudates were obtained from patients with diabetic ulcers of the foot following standard procedures. They were processed for the identification of the infective agent in the Department of Microbiology, Kerala medical college & Hospital, Palakkad

Blood sugar level

Blood sugar levels mg% -- number of Cases

<150 – 18, 150-200---42, 200-300--31 >300—9

18 out of 100 cases developed foot ulcers in spite of near normal blood sugar levels

History of onset of ulcer

Only 37 out of 100 patients gave the history of some form of injury and the rest informed that the ulcer has started spontaneously

Cases with wagner's grading of ulcers

More than half of the cases(52) presented with ulcers of grade – II. Grade 1 = 18,

Grade 3 =27, Grade 4= 0

Distribution Of Risk Factors In Cases

76 cases out of 100 were having peripheral Neuropathy and 70 cases symptoms of arteriopathy. 11 of the total patients got foot infection without any known risk factor.

Frequency Of Isolates In Various Wagner's Grades

Monomicrobial infections are more in grades–I &II and polymicrobial infections are more in grades– III & IV.

Frequency Of Isolates In Urban / Rural areas.

Only 11.54% of urban cases were polymicrobial where as 60.81% of rural cases were polymirobial infections.

Table 1: Sample collection - cases

Sample were collected from septic ward more patient 62 cases

Department	No of cases
Septic ward	62
Surgery	29
Endocrinology	9
Total	100

Table 2: SEX wise distribution cases

The male to female ratio was 2.03 in our study

Males	67
Females	33
Total	100

Table 3: Age wise distribution cases

The ulcers occured over a wide range of age from 16 years to 78 years with a mean age of 47 years.

64 out of 100 cases were between the ages of 45-65 years. There was only one case below 25 years of age and 3 cases above 75 years.

Age group	No of cases
<25Y	1
26-35	9
36-45	15
46-55	28
56-65	36
65-75	8
>75y	3
Total	100

Table 4: Duration of diabetes in the study group

Out of 100 patients 15 are unaware of the disease and diagnosed at the time of admission. 32 reported the duration of less than 5 years, 33 reported a duration of 5-10 years and 20 had the history of diabetes for more than 10 years.

Duration	No of cases
Not known before	15
<5y	32
5-10y	33
>10y	20
Total	100

Table 5: Glycaemic Control

Majority of the cases were under insulin treatment

Method	No of cases
Insulin	71
Oral hypoglycemics	29
Total	100

Table 6: Bacterial isolates from cases

Pseudomonas aeruginosa was the most common organism isolated and constitutes 32.43% of the total isolates.

Staphylococcus aureus was the second most common organism with 34 isolates and forms 22.97% of the total isolates.

Escherichia coli 32 (21.6%) Klebsiella spp 18 (12.16%) and Proteus spp 15 (10.13%) forms the rest of the isolates. Acinetobacter spp was isolated in one case

	No of cases	No of isolates
organism isolated in pure: Pseudomonas aeruginosa	23	23
Staphylococcus aureus	15	15
Escherihia coli	2	2
Klebsiella	9	9
Proteus	3	3
Organism isolated in combination : Pseudomonas + Escherichia Coli	14	28
Escherichia coli + Staph aureus	10	20
Escherichia coli + Proteus	6	12
Pseudomonas + Staph aureus	5	10
Pseudomonas + Proteus	3	6
Escherichia-coli + Klebsiella	3	6
Klebsiella +staph aureus	2	4
Pseudomonas + Klebsiella	2	4
Proteus +Staph aureus	1	2
Proteus + Klebsiella	1	2
Acinetobactor + Klebsiella	1	2
Total	100	148

*Antibiogram of staphylococcus aureus (*N = 34)

Staphylococcus aureus is showing highest sensitivity to Roxithromycin (26) followed by Cefaperazone + Sulbactam (23) combination and Amikacin (20). The organism is showing lowest sensitivity to cloxacillin (7) and pencilin G (5) among all the antibiotics tested.

Antibiogram of gram negative bacilli

Out of the seven antibiotics tested gram negative bacilli are showing highest sensitivity to amikacin followed by cefaperazone + sulbactam combination, Roxithromycin and Gentamycin. All the gram negative bacilli are showing least sensitivity to cloxacilin.

- 35 pseudomonas isolates were sensitive to cefaperazone + sulbactam followed by Roxithromycin (34) and Amikacin (32)
- 26 Isolates of E.coli were sensitive to Cefaperazone +Sulbactam followed by Amikacin (22) and Roxithromycin (22)
- 14 isolates of Klebsiella were sensitive to Amikacin followed by Roxithromycin (11) and cefaperazone + Sulbactam.
- 12 Isolates of Proteus were sensitive to Amikacin followed by Roxithromycin (9) and Cefaperazone + Sulbactam (8).

Single Acinetobactor isolate obtained was sensitive to cefaperazone + sulbactam Amikacin and gentamycin.

Out of the 15 Pseudomonal isolates that were subjected to Screening of Amp – C β lactomase by disc antagonism test only 4 isolates showed blunting of Cefotaxime zone of inhibition adjacent and to Cefoxitin and were considered screen positive.

Discussion

The present study was conducted to establish the bacteriology and antibiotic susceptibility of diabetic foot infections. Samples were collected from 100 cases admitted in septic ward, surgery and departments.

Out of 100 cases studied 67 were male and 33 were females with a male to female ratio of 2.03.

This correlates with the ratio of 1.95 by Viswanath et al. Chennai in 2002, 1.89 by C. Anandi et al Chennai 2004. Vijaya et al in 2000 reported a male to female ratio 2.63 from Bengaluru and Ekra Bansal et al. in 2008 presented a ratio of 3.68.

The mean age of patients with diabetic foot infections from the studies are over the world varies from 43 years to 57 years. The present study includes patients from 16-78 years with a mean age of 47 years Syed Md Alavi from Karachi, Pakistan Reported same value in 2007.

Peripheral neuropathy is commonly associated with diabetic infections and is one of the risk factor for the development of foot ulcers. In the present study 76 out of 100 cases presented with the signs and symptoms of neuropathy. This was same as Given by Ekta Bangal et al Chandigarh in 2008, Ravisekhar et al Delhi in 2006. and higher than Shanker et al., Chennai (56%) in 2005.

Diabetic foot infections are usually Polymicrobial in nature and always yield more than one isolate for sample on the whole. In the present study a total of 148 isolates were obtained from 100 cases making the number of isolates per sample 1.48. This was very close to 1.42 by Viswananth et al in Chennai in 2002 and 1.52 by Ekta. Bansal et al in 2008 from Chandigarh, but Citran. M et al. USA in 2004 reported 2.7 and N.A Pathera in 1998 as 3.07 from Mumbai.

Gram negative bacilli forms 77% of the total isolates. This was in contrast to the predominant isolation of Gram positive cocci (80%) by E.J. Goldstein et al. California. Hartmen Mecutem A et al., in 2004. observed 64% showing increasing trend of Gram negative bacilli in Diabetic foot infections. Pathare NA et al in 1998 from Mumbai reported 50% of Gram positive cocci in the first two grades and reduced to less than one fourth in the last two grades. Vijay D et al., Bengaluru (2002) 65%, Sasikala R et al., Pondicherry (2005) 77.94%, Ekta Bansal et al Chandigarith (2008) 77%, the last two being almost same as the present study.(Figure 1) .

Pseudomonas aeruginosa was the most common organism isolated and constitutes 32.43% of the total isolates. This correlates with the studies from various Geographical areas as 22% by Robert G et al, USA in 2002, 28% by Shankar EM et al. in 2005, 21.67% by Ekta Bansal, Chandigarh in 2008. Our study supports the conclusion by Shankar EM et al. from Chennai that recovery of multidrug resistant Pseudomonas aeruginosa is of serious concern, the first person to report this from South India.

Staphylococcus aureus was the second most common organism with 34 isolates and forms 22.97% of the total. This differs widely from 76% by E.J. Goldstem et al. USA in 1996 and 77% by Dang C.N. et al UK in 2003. but correlates with 24.5% by citron M et al., USA in 2004, 19% by Robert G et al. USA in 2002, 18.3% by Viswanath et al Chennai in 2002, 20% by Yoga R et al. Malaysia in 2006, 21.17% by Sasikala et al, Pondichery in 2005 and 18.88% by Ekta Bansal et al Chandigarh in 2008.(Figure 4).

More than 50% of Gram negative bacilli are sensitive to Amikacin, cefaperazone +sulbactam, Roxithanycin and Gentamycin. Amikacin was effective against 70.79% of Gram negative bacilli that was less when compared to 88.46% by Dushyanth Singh Uttaranchal 2007 and 81.14% by Robert E, USA, 2002. It was more than 52% sensitivity reported by Ravisekhar et al 2006, and 33.4% by Sasikala 2005.

Cefaperazone + Sulbactam was showing 69.91% effectiveness in the preset study and was less than 90.66% by Ravisekhar AIMS, 2006 and 97.5% Robert G, USA .

Gentamycin was sensitive against 53.09% of Gram negative bacilli which coincides with 54.4% by Robert G USA in 2002 Ciprofloxacin was effective against 36.25% bacteria which was 52% by Ravisekhar et al in 2006. Cephalexin was effective against 35.35% of Gram negative bacilli for which Dushyant Singh, Uttaranchal in 2007 reported 54.09% sensitivity. Roxithromycin was showing 67.25% effectiveness in the present study.(Figure 6)

Pseudomonas aeruginosa is showing maximum sensitivity to cefaperazone + sulbactam (72.91%) which was less than 93.5% reported by Robert E, USA, 2002 and 88.8% by Ravisekhar et al., New Delhi, 2006. The sensitivity to Roxithromycin was 70.83%. The third most effective antibiotic against Pseudomonas was Amikacin with 66.07% sensitivity which was more than 55.5% by Ravisekhar et al., 2006 and 33% by Sasikala et al., Pondichery but less than the studies of Robert E, 2002 (78.95%) Yoga R, Malasya (100%) and C. Anandi et al. 2004 (90%). The percentage sensitivity of Gentamycin and Ciproflaxcin were 52.08% and 31.25% in comparison to Robert E et al. (33.33% and 62.5%) Yoga R et al. (50% and 100%) and C. Anandi et al (90% and 90%). Cephalexin was effective in 43.75% of isolates which was 54.09% in 2007 by Dushyant Singh. Cloxacillin was the least effective antibiotic (4.1%) against Pseudomonas that was 0% in Saudi in 2007 given by Syed – Md Alavi. (Figure 7).

In the present study Pseudomonas aeruginosa was the predominate isolate with resistance to more than 50% of drugs. The 15 Pseudomonas isolate screened for Amp-C β lactamase by disc antagonism test. Showed 4 screen positive isolates Comparison with other studies is difficult to do, since the patient population is different. The prevalence was 22% in SS Hospital, Varanasi, compared to earlier studies in India that were 17.3% in Kolkota and 20% in Aligarh. As we found 4 out of 15 isolates as screen positive there is significant need to conduct a thorough study on the prevalence of Amp-C β lactamase in Pseudomonas aeruginosa in Mangode, Palakkad distric

Conclusion

- The present study was conducted to know the bacteriology and antibiogram of diabetic foot infections in and around Mangode, Palakkaddistrict.
- Out of 100 cases studied 67 were male and 33 were females with a male to female ratio of 2.03.
- The present study includes patients from 16-78 years with a mean age of 47 years.
- 76 Out of 100 cases presented with the signs and symptoms of neuropathy.
- A total of 148 isolate were obtained from 100 cases and the number of isolates per sample is 1.48.
- Pseudomonas aeruginosa was the most common organism isolated and constitutes 32.43% of the total isolates.
- Staphylococcus aureus was the second most common organism with 34 isolates and forms 22.97% of the total isolates.
- Escherichia coli 32 (21.6%) Klebsiella spp 18 (12.16%) and Proteus spp 15 (10.13%) forms the rest of the isolates. Acinetobacter spp was isolated in one case.
- The antibiotic sensitivity testing shows Amikacin, cefaperazone + sulbactam, Roxithromycin as the most effective antibiotics against Gram negative bacilli.
- Staphylococcus aureus is showing highest sensitivity to Roxithromycin followed by cefaperazone + sulbactam combination.
- 15 Isolates of Pseudomonas aeruginosa that were obtained from February 2008 were subjected to disc antagonism test for the detection of Amp – C β lactamase and 4 of them were screen positive.
- This finding may be taken as base line for further evaluation of prevalence Amp-C β lactamase production in this area.

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Original Article

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Clinical profile of cases of neonatal septicemia

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Abstract

Introduction: Neonatal septicemia is a clinical syndrome characterized by a nonspecific signs and symptoms in association with bacteremia that occurs in the first month of life. It is an important cause of morbidity and mortality among neonates in India with an estimated incidence of approximately 4% in intramural live births. Blood culture remains the gold standard for the diagnosis of neonatal septicemia. *Methodology:* Patients presented to department of pediatrics (NICU), were examined clinically by pediatricians and 122 cases of neonatal septicemia were identified on the basis of the signs and symptoms and were included for the study. *Results:* The distribution of 122 suspected cases of neonatal septicemia studied according place of birth was, 106 (86.88%) babies were inborn followed by 16 (13.12%) outworn babies. Of 16 outworn, 14 (11.48 %) neonates were born in the hospital and 2 (1.63%) babies had born in ambulance. *Conclusion:* In the present study, among 122 cases, 41(33.30%) were preterm babies, 80 (65.59%) were term babies and 1 (0.81%) neonate was found to be post term.

Keywords: Neonatal Septicemia; EOS; LOS.

Introduction

Sepsis in EOS and LOS manifests as clinical syndromes such as generalized sepsis, meningitis and respiratory symptoms. Asymptomatic bacteremia is a known characteristic feature in patients, in particular, with EOS.

The patients generally present with irritability, lethargy, temperature instability, poor feeding, vomiting, poor perfusion and hypotension. Patients with meningitis may present with seizures, apnoea, neck rigidity and altered sensorium. Respiratory symptoms can range in severity from mild tachypnoea, grunting, to respiratory distress and failure. Persistent pulmonary tension of the newborn can also accompany sepsis. Severe sepsis patients may present with disseminated intravascular coagulation (DIC) with purpura and petechiae [1].

In fact, French midwives and obstetricians have made the major contributions in beginning the history of neonatology and not the pediatrician even after specialized care of infants was introduced to the United States, however it was observed that some of the most early practitioners and researchers were obstetricians and anesthesiologists. Another interesting evidence of the early history of neonatology was the existence of "incubator baby side shows" at global fair in America over a 40 years period from 1898, also by Transmissisippi exposition in Omahato and New York world's fair in 1939. These made possibility of regionalized intensive care for hundreds of new born by bringing down the rate of mortality [13].

The first scientific epidemiological study carried out by Ignaz Semmelweis suggested how diseases were transmitted and measures to interrupt transmission [2].

IgnazSemmelweis who was a Hungarian obstetrician observed the pregnant women dying of puerperal sepsis (child bed fever) during labour in his hospital and found that disease was more prevalent in the ward handled by medical students suggesting the mode of transmission which involved medical students and the source of contagion could be cadavers on which the medical students had previously performed autopsies. In 1847, Semmelweis directed his staff and students to wash their hands with chlorine water before entering the maternity ward. This simple hygienic practice could result in the reduction of mortality associated with childbed fever [3].

Providing of neonatal care facilities, mainly the primary and some secondary care began in India during 1960s. However Neonatal intensive care unit(NICU) concept in India began to make its appearance only in the 1980s in a selected teaching institutions across the country when few neonatologists decided to import/implement the western modes of NICU facilities but still in the early 1990s there were very small number of NICU facilities available across the country [3].

Neonatal septicemia is a clinical syndrome characterized by a nonspecific signs and symptoms in association with bacteremia that occurs in the first month of life. It is an important cause of morbidity and mortality among neonates in India with an estimated incidence of approximately 4% in intramural live births. Blood culture remains the gold standard for the diagnosis of neonatal septicemia [4,5].

Neonatal septicemia is classified as "EOS" if it occurs within the first week of life and as "LOS" if occurring after the first week until the end of the neonatal period as noted above. EOS is conventionally regarded as maternally-acquired, with causative organisms such as *Escherichia coli* (*E. coli*) and GBS usually found in the maternal genital tract, whereas LOS is considered environmental in origin either hospital or community acquired. Commonly implicated organisms in hospital acquired are CONS, *Staphylococcus aureus* (*S.aureus*) and Gram negative bacilli (GNB) such as *Klebsiella* and *Pseudomonas* species [6].

Kaistha and colleagues [7] in their study, suspected sepsis - when neonates had systemic signs such as lethargy, chest retraction, grunting, abdominal distension, tachycardia, hypothermia in presence or absence of maternal risk factors such as chorioamniotis, prolonged rupture of membranes(>24hrs), diarrhea, fever or urinary tract infection.

Camacho-Gonzalez and coworkers[8] in their review enlisted maternal risk factors like GBS colonization, chorioamniotis, prolonged rupture of membranes, multiple pregnancies, preterm delivery and neonatal risk factors such as prematurity and low birth weight for the occurrence of neonatal septicaemia. A total of 46(19%) mothers of septicaemic neonates had prolonged or obstructed labour, 34(14%) had prolonged rupture of membranes, 25(10%) had PROM where as pre-eclampsia was reported in 13(5%).

Methodology

Patients presented to department of pediatrics(NICU), were examined clinically by pediatricians and 122 cases of neonatal septicemia were identified on the basis of the signs and symptoms and were included for the study. This is followed by collection of blood for culture after obtaining informed expressed written consent.

A total of 122 neonates clinically suspected of neonatal septicaemia reported to Hospital, were examined during a study period and the criteria were as outlined below.

Inclusion criteria

1. Clinically suspected cases of neonatal septicaemia.

Exclusion criteria

1. Neonates clinically suspected of septicaemia but had received antibiotics were excluded from the study.

1-2mL of blood was collected from the peripheral veins following all standard aseptic precautions as per CLSI guidelines. The collected blood specimen was immediately inoculated onto 5mL(when 1mL was obtained) or 10mL(when 2mL was obtained) of liquoid broth(BHI broth with SPS) culture medium and mixed gently immediately.

Results

Table 1: Age and sex distribution of 122 neonatal septicaemia cases studied

Age (Days)	Male No (%) of cases	Female No(%) of cases	Total No(%) of cases
1	40(32.79)	37(30.32)	77(63.11)
2	03(2.45)	06(4.91)	09(7.37)
3	07(5.73)	05(4.10)	12(9.83)
4	07(5.73)	00(00)	07(5.73)
6	00(00)	03(2.45)	03(2.45)
7	00(00)	01(0.81)	01(0.81)
8	00(00)	01(0.81)	01(0.81)
10	02(1.63)	01(0.81)	03(2.45)
12	01(0.81)	00(00)	01(0.81)
14	00(00)	02(1.63)	02(1.63)
15	00(00)	01(0.81)	01(0.81)
21	00(00)	01(0.81)	01(0.81)
24	00(00)	01(0.81)	01(0.81)
26	00(00)	02(1.63)	02(1.63)
27	00(00)	01(0.81)	01(0.81)
Total	60(49.19)	62(50.81)	122(100)

Age of neonates was from 1day to 27days, the mean age of patients being 2.77 days with SD4.44 days. Out of the 122 neonates with clinical suspicion of septicaemia studied, 109 (89.3%) belonged to EOS and 13 (5.7%) to LOS. Among 122 neonates, females were 62 (50.81%), whiles males were 60 (49.19%). Among females, mean age observed was 3.44 days with SD 5.49 days, similarly in males, mean age was 2.08 days SD being 2.8 days.

Table 2: Depicting gestational age of 122 neonatal septicaemia cases studied

Gestational age	No(%) of cases
Preterm	41(33.60)
Term	80(65.59)
Post term	01(0.81)
Total	122

Among 122 suspected cases of neonatal septicaemia, 41(33.30%) were preterm babies, 80 (65.59%) were term babies and 1(0.81%) neonate was found to be post term.

Table 3: Distribution of 122 neonatal septicaemia cases studied according to the place of birth

Place of birth	No(%) of cases
Inborn(BIMS Hospital)	106(86.88)
Outborn(16(13.12)	
Hospital	14(11.48)
Ambulance	2(1.63)
Total	122

The distribution of 122 suspected cases of neonatal septicemia studied according place of birth was, 106 (86.88%)babies were inborn (BIMS, Hospital) followed by 16(13.12%) outworn babies. Of 16 outworn, 14 (11.48 %) neonates were born in the hospital and 2(1.63%) babies had born in ambulance.

Table 4: Distribution of 122 neonatal septicaemic cases studied according to the mode of their birth.

Mode of birth	No(%) of cases
Spontaneous vaginal	100(81.97)
Assisted vaginal	1(0.81)
Cesarean section	21(17.22)
Total	122

Among 122 suspected cases of neonatal septicaemia studied, 100(81.97) neonates took birth by spontaneous vaginal followed by by cesarean section in 21(17.22%) and birth by assisted vaginal was seen in 1(0.81%) case.

Table 5: Distribution of birth weight recorded in 122 neonatal septicaemic cases studied

Birth weight	No(%) of cases
Normal weight (2.5 kg-4 kg)	59(48.36)
Low birth weight (1.5 kg- ≤2.4 kg)	53(43.44)
Very low birth weight (1 kg- ≤ 1.4kg)	9(7.37)
Extremely low birth weight (Less than 1 kg)	1(0.81)
Total	122

Among 122 cases, 59 (48.36%) neonates had normal weight while 53 (43.44%) patients had LBW followed by 9 (7.37%) cases with very low birth weight and 1 (0.81%) case exhibited extremely low birth weight.

Table 6: Distribution of maternal risk factors seen among 37 mothers (122 neonatal septicaemic cases studied)

Risk factors	No(%) of cases
Prolonged rupture of membranes	04(10.81)
Premature rupture of membranes	03(8.10)
Foul smelling / meconium stained liquor	06(16.21)
Multiple pregnancies	12(32.43)
Pregnancy induced hypertension	08(21.62)
Others(04) 1. Hypertensive 2. Prolonged labour	 01 (2.70) 03 (8.10)

A total of 37 (3.01%) neonates with septicaemia had maternal risk factors. Multiple pregnancies was the predominant risk factor in 12 (32.43%) cases. However, pregnancy induced hypertension was seen in 8 (21.62%) followed by foul smelling / meconium stained liquor in 6 (16.21%), prolonged rupture of membranes in 4 (10.81%), PROM in 3(8.10%)cases and other factors, for instance, previously diagnosed as hypertensive and prolonged labour was seen in 1 (2.70%) and 3 (8.10%) patients respectively.

Table 7: Distribution of clinical presentation noticed in 122 neonatal septicaemic cases studied

Clinical presentation	No [in various combinations] (%) of cases
Hyperthermia/hypothermia	2(1.63)
Tachypnoea/apnoea	82(67.21)
Pathological jaundice	1(0.81)
Refusal for feeds	43(35.24)
Loss of activity	45(36.88)
Seizures	11(9.01)

Among 122 neonatal septicemic cases studied, tachypnoea/apnoea was predominant clinical symptom which was documented in 82 (67.21%) neonates, 45 (36.88%) cases had loss of activity followed by refusal for feeds in 43 (35.24%) patients. However, seizures were determined in 11 (9.01%) cases, hyperthermia/hypothermia and pathological jaundice was seen in 2 (1.63%) and 1 (0.81%) neonates respectively.

Discussion

The present study conducted on 122 neonatal septicemic cases, EOS comprised of large number of cases that was seen 98 (89.34%) neonates which is similar to other studies carried out elsewhere. Naher and Khamael [9] documented EOS in 29(58%) neonates of 50 cases studied. Raha *et al.* [5] recorded EOS in 45(70.3%) of the total 55 cases studied. Tallur *et al [10]* reported incidence of EOS in 202 (83.47%) among 242 cases studied. Incidence of EOS which was observed more commonly in our study can be attributed to vertical transmission during birth, immature immune responses and can also be explained by the maternal risk factors such as PROMS, multiple pregnancies.

Our study showed lesser incidence of septicaemia in preterm neonates accounting to 41(33.60%) babies compared to term 80 (65.59%) babies during the study period. LBW was noted in 53 (43.44%) neonates which is a common neonatal risk factor observed in numerous studies [11]. In a study conducted by Raha*et al. [11]*, preterm was reported in 71.88% whereas LBW was seen in 65.63% of total neonates suspected for septicaemia. Tallur*et al [10]* recorded preterm in 96 (39.67%) and LBW in 132 (54.55%) babies among total neonatal septicaemic cases studied and stated that the prematurity and LBW as the most important predisposing factors in neonatal septicaemia because of the immature immune system development. Our study almost correlates with the study carried out by Tallur *et al. [10]* but there is significant difference seen when compared with the observations of Raha *et al. [11]* in their study, Number of preterm labour and LBW babies is directly proportional to the frequency of maternal risk factors observed. Lower incidence of preterm birth and LBW babies could be due to less number of maternal risk factors found in the present study.

In a study conducted by Tallur *et al. [10]*, most common maternal risk factors observed were prolonged/ obstructed labour in 46(19.01%) mothers, prolonged rupture of membranes in 34 (14.05%) and PROM in 25 (10.33%) mothers. Camacho-Gonzalez and colleagues [8] in the recent review enlisted maternal risk factors like group B streptococci colonization, **chorioamniotis,** prolonged rupture of membranes, multiple pregnancies for neonatal septicaemia and also emphasized the role of maternal risk factors in neonatal infection. However, in our study multiple pregnancy was most common maternal risk factor which was seen in 12 (32.43%) mothers followed by PIH in 8 (21.62%) and Foul smelling / meconium stained liquor in 6 (16.21%) mothers.

Tallur*et al. [10]* although observed diverse clinical manifestations. Jaundice in 64(26.5%) and apnoea in 54 (22.3%) neonates were noticed as main features in their study conducted for 242 newborns with septicaemia. In the present study, tachypnoea/ apnoea was most common clinical symptom which was documented in 82 (67.21%) neonates, 45 (36.88%) cases had loss of activity. Tallur*et al. [10]* reported a total of 35 (14.5%) neonates suspected for septicaemia with foci of infection like umbilical sepsis, pyoderma, abscess and conjunctivitis.

Conclusion

However, interestingly there were no foci of infection found in any of the neonates in our study which could be because of clean, safe deliveries and other aseptic precautions taken during labour.

Table 8: Shows neonatal risk factors detected in different studies among neonatal septicaemic cases studied.

Authors and reference	Preterm No (%) of cases	Low birth weight No (%) of cases
Rahaet al11	46(71.88)	42(65.63)
Talluret al 10	96(39.67)	132(54.55)
Present study	41(33.60)	53(43.44)

In the present study, among 122 cases, 41(33.30%) were preterm babies, 80 (65.59%) were term babies and 1(0.81%) neonate was found to be post term.

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Case Report

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Enterobius vermicularis (Pinworm infection) in HIV infected patient with chief complain of uncontrolled diarrhoea

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Abstract

Enterobiasis is a uncommon nematode infestation that can been caused uncontrolled diarrhoea in HIV infected patient. It is an important tropical infection that can affect the patients at any sexes and age groups. The present of enterobiasis in HIV infected patients is of interest. In this short article, the authors summarize on Enterobiasis in HIV infected patients.

Keywords: HIV; Enterobiasis; Diarrhoea.

Introduction

Enterobius vermicularis, often referred to as pinworm, is an intestinal nematode which commonly infects children [1]. Transmission of *E. vermicularis* eggs occurs through the fecal-oral route, with eggs being directly inoculated from the fingers into the mouth. The migration of the female worm to the anus causes pruritus, which is the most common symptom of pinworm infection [2,3]. We have reported the *Enterobius vermicularis* infection in to Adult patient suffering from HIV infection with chief complaint of uncontrolled diarrhoea.

Case Report

A 41 yrs old male patient, who belong to Western Part of India having H/O Retrovirus illness detected in year 2007. Patient was on treatment of ART (AZT + ZTC + NVP) from 2009 to 2011. At that time complain of low grade fever, generalise weakness and loss of appetite. Patient has history of Pulmonary Koch & AKT taken for Total 12 months

Patient CD4 count, 70/cmm in year Nov 2011, 108/cmm in year Feb 2012, 20/cmm in May 2012, 168/cmm in March 2013, 106/cmm in Dec 2013, 131/cmm in March 2014, 63/cmm in May 2014, 127/cmm in Dec 2014, 74/cmm in Feb 2015, 62/cmm in July 2015, 31/cmm in Nov 2015, 216/

cmm in Feb 2016, 290/cmm in May 2016, 91/cmm in Nov 2016.

Patient HIV viral load count was 15340800 (IU/ml) in May 2014, 14370 (IU/ml) in Nov 2015. Patient HIV drug resistance genotyping show -

Patient was on Following ART regime

2009 to 2011 – AZT +ZTC+NVP

2012 to 2016– AZT+RTV+TNF+FTC

Patient came with chief complaints of loss of appetite, coughing, dysphagia, abdominal discomfort and oral thrust since one month, watery diarrhoea since 15 days. Gradually diarrhoea worsen. Patient has no complain of pruritis. His CD4 count was 23/cmm. Stool sample were sent for Microbiology work up – Bacterial Culture, Modified Z.N Stain, Wet preparation for parasite and occult blood. Stool was negative for *Cryptosporidium* species, *Blastocystis spp., Isospora belli*, *Giardia lamblia*, *Entamoeba histolytica and Strongyloides stercoralis*, Bacterial culture grown *Escherichia coli* and yeast (considered as Normal flora). Ocult blood was positive and mucus were present. Stoll was positive for plenty eggs of *Entrobius vermicularis*. USG abdomen shows fluid in right colon, sign of mild colitis and mild haepatomegaly.

After having report of eggs of *Enterobius vermicularis*, patient treated with Oral Mebendazole 100mg stat and oral Albendazole 400mg twice a day for 3 days. Gradually diaerrhoea controlled and patient become stable with in 5 days of treatment.

Discussion

Chronic infection with parasite are common in India. *Enterobius vermicularis*, often referred to as pinworm, is an intestinal nematode which commonly infects children throughout the world. Transmission of E. vermicularis eggs occurs through the fecal-oral route, with eggs being directly inoculated from the fingers into the mouth. Fomites may also play a role in transmission. The eggs are infective shortly after being laid, making autoinfection a common route of intestinal infection. Following ingestion, the embryonated eggs hatch in the small intestine and develop into adult worms that reside in the cecum, appendix, colon, and rectum. Male and female worms mate in the human intestinal tract, and the gravid female worm migrates to the anus to lay partially embryonated eggs on the perianal and perineal surfaces. The migration of the female worm to the anus causes pruritus, which is the most common symptom of pinworm infection (2, 3). Less commonly, the presence of adult worms in the appendix can lead to obstruction, inflammation, and resultant appendicitis (4, 5). Rarely, the adult worms can become lodged in the intestinal mucosa and cause intestinal abscess (1)

HIV infection has been modifying both the epidemiology and outcome of parasite infections. Opportunistic parasitic infection in HIV infected patient is common. The common opportunistic pathogen in HIV infected patients are *Cryptosporidium* species, *Blastocystis spp., Isospora belli*, *Giardia lamblia*, *Entamoeba histolytica and Strongyloides stercoralis.(6)*

The reported prevalence rates of Entrobius vermicularis in HIV infected patients from Kenya, Ehiopia, Vietnam, Congo and South Africa are equal to 1.9%, 1.3 %, 0.9 5, 0.6 %, and 0.6 %.(7)

In this case the clinical presentation of Enterobiasis was atypical. Entrobiasis usually occurs to children and the chief complains are pruritis in anal region. In this case the chief complaint was uncontrolled diarrhoea.

Conclusion

Enterobiasis can be seen in the patients with HIV infection. This parasitic infestation might be silent or produce severe clinical problems. The concern on this parasitic infestation among HIV infected patient is needed. No doubt that if there is existence of enterobiasis, regardless of symptom, the antiparasitic drug should be provided to the HIV infected patients for prevention of unwanted further complication. Focusing on the use of antiparasitic drug, ARVT and antituberculosis drugs were observed to be better tolerated in HIV infected patients.(8)

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