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Journal of Microbiology and Related Research

JMRR

January - June 2019
Volume 5, Number 1

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Original Article

Journal of Microbiology and Related Research

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.1

Bacteriological Profile and Antibiogram of Isolates from Burn Ward in A Tertiary Care Hospital

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Received on 17.01.2019, **Accepted on** 04.02.2019

Abstract

Introduction: Burn patients are at a risk of infection because of their destroyed skin barrier, suppressed immune system compounded by prolonged hospital stay and invasive therapeutic & diagnostic procedure. Despite various advances in infection control measures like early detection of causative agent and use of newer and broad spectrum antibiotics, management of burn septicemia still remains a big challenge. *Aim and Objective:* To study prevalence of various aerobic bacterial isolates among burn wound infection and to study their antimicrobial susceptibility pattern. *Material and Methods:* During study period of Jan- June 2017, a total of 369 pus samples were received and inoculated on different media as per standard protocol. Isolate were identified and antimicrobial susceptibility testing was done as per CLSI guidelines (2016). *Results and observation:* Out of 369 samples, 241 (65.31%) samples were from female and 128 (34.69%) from male. Among these 209 (56.64%) samples were having single isolate and in 125 (33.88%) samples, two types of bacteria were isolated. Out of 459 isolates, 133 Gram positive cocci and 326 Gram negative bacilli were isolated. Among various bacterial isolates *Pseudomonas aeruginosa* was most commonly isolated bacteria followed by *Staphylococcus* spp. and *Klebsiella pnemoniae.* Most of the isolates were resistant to routinely used antimicrobial agents. *Conclusion:* P. *aeruginosa* and *S. aureus* are the leading cause of infection in burn patients and isolation of multidrug resistant organism should be considered as a serious risk in burn unit. Early identification of infection caused by multidrug resistant organisms might help to modify treatment and outcome in burn patient.

Keywords: Burn Wound Infection; *P. Aeruginosa; S. Aureus* and Multidrug Resistance.

How to cite this article:
Gajbhiye MR, Chincholkar VV, Mangalkar SM et al. Bacteriological Profile and Antibiogram of Isolates from Burn Ward in A Tertiary Care Hospital. J Microbiol Relat Res. 2019;5(1):5-8.

Introduction

Burn wound infection is one of the most common cause of morbidity and mortality [1]. Burn patients are at a risk of infection because of their destroyed skin barrier, suppressed immune system compounded by prolonged hospital stay and invasive therapeutic & diagnostic procedure [2].

It has been estimated that approximately 75% of all death following burns are related to health care associated infections [3] which occurs due to infection by the organism of patients own flora, colonizers of the environment or from health care personnel [4]. Initially Gram positive organism derived from skin commensal colonize the wound bed, followed later by Gram negative organism and yeast. *Staphylococcus* spp. and *P.aeruginosa* are most frequently isolated microorganisms [5].

The pattern of infection differs from hospital to hospital and the bacterial flora of infected wound may change considerably during healing. Despite various advances in infection control measures like early detection of causative agent and use of newer and broad spectrum antibiotics, management of burn septicemia still remains a big challenge [6]. The worldwide emergence of antimicrobial resistance among wide variety of burn wound pathogen particularly health care associated isolates, limits the available therapeutic option for effective treatment of burn wound infection [7].

Therefore present study was undertaken to know the antimicrobial susceptibility pattern of various bacterial isolates recovered from burn patients which will help in instituting empirical therapy and minimize irrational use of antimicrobial agents.

Aim and Objectives

To study prevalence of various aerobic bacterial isolates among burn wound infection and to study their antimicrobial susceptibility pattern.

Material and Method

The study was done for a period of six month from Jan-June 2017. During six months period a total of 369 pus samples were received in the department of Microbiology from burn patients. Gram stain was done, followed by inoculation of the sample on blood agar and MacConkey agar [8]. The inoculated media were then incubated overnight at 37^0C aerobically and identification of the organisms was done by Gram stain, colony morphology and biochemical reactions as per standard protocol [9]. The antimicrobial susceptibility testing was carried out by Kirby Bauer disc diffusion method according to Clinical Laboratory Standard Institute guidelines [10] (2016) using commercially available antimicrobial discs procured from Hi-Media Laboratories Pvt. Ltd.

Result and Observations

During a study period, a total 369 samples were received from burn patients, out of which 241 (65.31%) were from female and 128 (34.69%) from male. Among 369 samples, 209 (56.64%) were having single isolate and in 125 (33.88%) samples, two types of bacteria were isolated and in 35 (9.48%) samples showed no growth in culture.

Table 1: Gender wise distribution of samples collected from patients (N=369)

Gender	Total no. of swab	Percentage
Male	128	34.69%
Female	241	65.31%

Out of 459 isolates, 133 Gram positive cocci and 326 Gram negative bacilli were isolated. Among various bacterial isolates *Pseudomonas Aeruginosa* was most commonly isolated bacteria followed by *Staphylococcus* spp. and *Klebsiella Pneumoniae*.

In our study, methicillin resistance was 94.87% among *Staphylococcus Aureus* and 75% in Coagulase negative Staphylococci. Most of the isolates were resistant to penicillin, ciprofloxacin and gentamicin and susceptible to vancomycin, linezolid and teicoplanin. D test was performed to see inducible clindamycin resistance which showed 68 isolates to have D test positive.

Most of Gram negative isolates were resistant to routinely used antimicrobial agents and even 39 out of 326 isolates were resistant to imipenam which is used as reserve drug for multidrug resistant organism.

Table 2: Distribution of bacterial isolates from infected burn wounds

Organism	Total (n= 459)	Percentage (%)
Pseudomonas aeruginosa	156	33.99
Staphylococcus aureus	117	25.49
Klebsiellapneumoniae	110	23.96
Escherichia coli	31	6.75
Proteus spp.	18	3.92
CONS	16	3.49
Acinetobacter spp.	11	2.40

CONS = Coagulase negative Staphylococcus

Table 3: Antimicrobial resistance pattern among Gram positive cocci

Antibiotics	*S. aureus* (N=117)	*CONS* (N=16)
Amikacin	59 (50.43%)	5 (31.25%)
Cefoxitin	111 (94.87%)	12 (75%)
Ciprofloxacin	92 (78.63%)	09 (56.25%)
Clindamycin	81 (69.23%)	09 (56.25%)
Erythromycin	84 (71.79%)	10 (62.5%)
Gentamicin	87 (74.35%)	11 (68.75%)
Netilmicin	51 (43.58%)	05 (31.25%)
Penicillin	117 (100%)	15 (93.75%)

Table 4: Antimicrobial resistance pattern in Gram negative isolates

Antibiotics	*P. aeruginosa* (N=156)	*Acinetobacter* (N=11)	*Klebsiella pnemoniae* (N=110)	*E. coli* (N=31)	*Proteus spp.* (N=18)
Amikacin	NT	NT	75 (68.18%)	19 (61.29%)	12 (66.66%)
Aztreonam	NT	NT	71 (64.54%)	19 (61.29%)	12 (66.66%)
Cefixime	NT	8 (72.73%)	78 (70.91%)	21 (67.74%)	12 (66.66%)
Ceftazidime	123 (78.85%)	8 (72.73%)	79 (71.82%)	22 (70.96%)	13 (72.22%)
Cefotaxime	NT	9 (81.82%)	90 (81.82%)	24 (77.42%)	13 (72.22%)
Ciprofloxacin	126 (80.77%)	9 (81.82%)	93 (84.54%)	25 (80.64%)	14 (77.77%)
Cefpodoxime	NT	8 (72.73%)	86 (78.18%)	24 (77.42%)	14 (77.77%)
Ceftizoxime	NT	9 (81.81%)	79 (71.82%)	23 (74.19%)	13 (72.22%)
Cefepime	117 (75%)	8 (72.73%)	78 (70.91%)	22 (70.96%)	13 (72.22%)
Gentamicin	126 (80.77%)	9 (81.82%)	82 (74.54%)	24 (77.42%)	14 (77.77%)
Netilmicin	NT	NT	84(76.36%)	23 (74.19%)	14 (77.77%)
Tobramycin	93 (59.61%)	7 (63.64%)	59 (53.64%)	15 (48.38%)	9(50%)
Piperacillin-tazobactam	109 (69.87%)	6 (54.54%)	66 (60%)	20 (64.51%)	11 (61.11%)
Cefoperazone	124 (79.48%)	NT	NT	NT	NT
Imipenam	23 (14.74%)	3 (27.27%)	10 (9.09%)	1 (3.22%)	2 (11.11%)

NT = Not Tested

Discussion

Burn injury is one of the more common and devastating forms of trauma in many areas of the world. Infection in burn patients is of major concern as it complicates overall management. Irrational and long term administration of oral and intravenous antibiotics could lead to development of antimicrobial resistance among the pathogens.

In our study, total of 369 samples were received from burn patients amongst which female to male ratio was 1.9:1, similarly in a study done by Rathod V S et al. (2017) [7] who showed that isolates from female (76.14%) were more than male (23.85%) while lower rate was seen in a study done by Dash et al . (2013) [2] & Rajeshwar et al. (2014) [11], who showed female : male ratio as 1.17:1 & 1:1.3 respectively. In India higher incidence of burn injuries among females may be related to inadequate precautions during cooking, wearing of loose sarees, inability to cope up with the physical and psychological stress of marriage and harassment from parents in law [2].

Among 369 samples, growth was seen in 334 (90.51%) sample and no growth in 35 (9.49%) samples which is similar to study done by Rathod V S et al. (2017) [6], who showed isolation of organism from 96.14% samples. Single organism was isolated from 209 (56.63%) and multiple isolates were found in 125 (33.87%) samples which is similar to study of Mohapatra et al. (2017) [4] where single isolates was seen in 43% and multiple isolate 32%.

Out of 469 isolates, 133 (28.97%) Gram positive cocci & 326 (71.03%) Gram negative bacilli were isolated which is similar to study done by Asati et al. (2017) [5] who showed isolation of Gram negative bacilli and Gram positive cocci as 76% and 24% respectively, while higher isolation of Gram negative bacilli (83.59%) was shown by Pooja et al. (2016) [1].

Among various bacterial isolates, *P.seudomonas aeruginosa* (33.98%) was most commonly isolated bacteria which is similar to study done by Pooja et al. (2016) (33.59%) 1, Mohapatra et al. (2017) [4] (27%), while higher isolation was seen in a study done by Dash et al. (2013) [2] (49.4%). Although *S.aureus* remains a common cause of early burn wound infection, *P.aeruginosa* from patient's endogenous gastrointestinal flora or moist environmental source is the most common cause of burn infection. The second most common isolate in our study was *Staphylococcus aureus* (25.48%) followed by *Klebsiella Pneumoniae* (23.96%) which is similar to study of Dash et al. (2013) [2] while in a study done by pooja et al. (2016) [1], *Klebsiella Pneumoniae* was second most common isolate.

In our study methicillin resistance was 94.87% among *S.aureus* and 75% among coagulase negative *Staphylococcus* which is much higher than study done by Mohapatra et al. (2017) [4] who showed prevalence of methicillin resistance as 20% and 40% respectively. In our study, all isolates were resistant to penicillin, 69.2% to clindamycin and 71.79% to erythromycin and coagulase negative *Staphylococcus* (CONS) were 93.75% resistant to penicillin, 56.25% to clindamycin and 62.5% to erythromycin. In our study erythromycin and clindamycin resistance was higher as compared to study done by Asati

et al. (2017) [5]. D test was done to see inducible clindamycin resistance which showed 54.70% *S.aureus* isolates and 25% CONS isolates D test positive, while in study done by Mohapatra et al. (2017) [4] 43% CONS shows positive D test. All isolates were sensitive to linezolid, vancomycin, teicoplanin similar to study of Pooja et al. (2016) [1] and Dash et al. (2013) [2].

In our study, most of the isolates of *P.aeruginosa* were resistant to commonly used antibiotics and 23 (14.74%) out of 156 isolates were resistant to imipenam which is higher than study of Dash et al. (2013) [2], Rathod et al. (2017) [7], and similar to study done by Pooja et al. (2016) [1] (15.90%). Among *Acinetobacter* isolates 3 (27.27%) out of 11 isolates of were resistant to imipenam which is lower as compared to study done by Pooja et al. (2016) [1] while in study done Dash et al. (2013)[2] all the isolates were sensitive to imipenam. All the isolates of *P.aeruginosa* and *Acinetobacter* spp. were sensitive to colistin.

In our study members of *Enterobacteriaceae* family were also resistant to most of the commonly used antibiotics and even 13 out of 159 isolates were resistant to imipenam which is less than study done by Pooja et al. (2016) [1] while in study done by Rathod V S et al. (2017) [7] & Rajeshwar et al. (2014) [11] all the isolates were sensitive to imipenam. In our study resistance pattern was higher than other may be due to improper and over use of antibiotics. It is also known that widespread use of broad spectrum antimicrobials in burn units may lead to acquisition of resistance and transformation to form new strains.

Conclusion

The present study conclude that *P. aeruginosa* and *S. aureus* are the leading cause of infection in burn patients and isolation of multidrug resistant organism should be considered as a serious risk in burn unit. Early identification of infection caused by multidrug resistant organisms might help to modify treatment and outcome in burn patient.

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.2

Comparison between Lactophenol Cotton Blue and Iodine Glycerol for Identification of Fungal Elements in Clinical Samples

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Received on 19.01.2019, **Accepted on** 07.03.2019

Abstract

Fungal infection is alarming phenomenon now a day because of increasing number of patients. Lactophenol Cotton Blue is widely used for staining, but there is a necessity to develop an alternate equipotent stain which can replace it due to its high level of tumorigenic and hazardous nature. iodine glycerol is better alternative for it. Iodine glycerol is safe, eco-friendly, good visual clarity, with better staining properties. Hence, we got here various clinical sampleswhichwere analysed simultaneously using Lacto-phenol Cotton Blue and Iodine Glycerol by using different techniques like teasing technique, slide culture technique and adhesive tape technique. Parameters like degree of transparency, visual clarity, resolution, contrast, staining characteristics like uniformity, formation of artefacts were analysed for better demonstration of fungal morphology. Iodine-Glycerol is a better alternative to Lacto-phenol Cotton Blue for the demonstration of fungal morphology in the clinical microbiological laboratories. It is eco-friendly, noncarcinogenic and much potent staining reagent. It is necessary to carry further research as there are no specific guidelines regarding the preparation of the Iodine-Glycerol staining reagent.

Keywords: LPCB, Iodine Glycerol; Lugol's Iodine; Fungal Morphology; Transparency; Staining Characters.

How to cite this article:
Seematai Prakash Katole, Halgarkar Charushila Sheshrao, Nilekar Sandeep Laxmikant. Comparison between Lactophenol Cotton Blue and Iodine Glycerol for Identification of Fungal Elements in Clinical Samples. J Microbiol Relat Res. 2019;5(1):9-12.

Introduction

For identifying filamentous fungi based on their characteristic morphological features, Microscopic observation of wet mounts is the most widely used method in clinical microbiology laboratories. most common technique used is lactophenol cotton blue tease method. Lactophenol cotton blue is preferred universally for its usage as a fixative, staining and mounting methods. The phenol component of lactophenol cotton blue is carcinogenic and hence it is imperative to search an alternate staining reagent. Hence, need arise for alternative safe and equally functioning fungal mount method.

Lugol's Iodine is a potent fungicide as it reacts with thiol groups of fungal enzymes and proteins and hence can functionally replace the phenol component in Lactophenol cotton blue. Although iodine solution in combination with chloral hydrate (Melzer's solution) is currently being used in clinical microbiological laboratories, it has never been used as a mounting method for microscopic identification of fungi (Baszkowski et al., 2006). Alsochloral hydrate is known to be a hazardous substance. The Iodine component in Lugol's Iodine stains the outer wall of fungus and can functionally replace cotton blue as a staining reagent.

Addition of 0.25% pure Glycerol to Lugol's

Iodine can potentiate the hygroscopic nature of the Glycerol-Iodine. Hence, we examined the possibility of using iodine-glycerol as an alternative to LPCB and to evaluate its usefulness for wet mount preparations for microscopic observation and identification of certain clinical isolates of filamentous fungi.

Materials and Methods

The present study was conducted from May 2017 to October 2017. Institutional Ethical committee approval was taken before conducting the study. Lactophenol Cotton Blue, Lugol's Iodine and pure Glycerol were purchased from Hi-Media.0.25 ml of pure Glycerol was added to 99.75 ml of distilled water to prepare 0.25% Glycerol. Equal quantities of 0.25% Glycerol and Lugol's Iodine were added to prepare the final stain Glycerol-Iodine. 64 clinical samples were processed. corneal scrapings, bits of tissue, nail clippings, hair plucks, sputum, bronchial washings, skin scrapings, etc were samples for fungal infection. skin scrapings, corneal scrapings, sputum etc were kept in 10% potassium hydroxide for 30 minutes to dissolve the cementing substance holding the keratinised cells followed by thorough analysis under low power field for the presence of fungal elements. nail clippings, bits of tissue etc were kept in 40% potassium hydroxide and left incubated overnight at 37^0C followed by analysis for fungal elements. nail clippings, bits of tissue etc were kept in 40% potassium hydroxide and kept in incubator overnight at 37^0C followed by analysis for fungal elements. along with this lactophenol cotton blue and glycerol-iodine mounting were done, Various criteria like the degree of transparency, staining characteristics, visual clarity and better demonstration of the morphology of the fungus under study are taken as measuring points to compare the efficacy of Iodine Glycerol with Lactophenol Cotton Blue stain.

Results and Discussion

The visual clarity and the degree of transparency were more with Iodine-Glycerol as compared to Lactophenol Cotton Blue. Contrast is reasonably good with Lactophenol cotton Blue. resolution is observed same with both. The staining characteristics like uniformity, clarity of the various morphological structures, lack of any artefacts due to the staining material on prolonged storage etc were better appreciated with Iodine – Glycerol than lactophenol cotton blue.

The observations in my study correlated with that of Vignesh et al., (2008) and Vacharavel, shamly et al., (2014).

We could not get adequate references due to dearth of information on the topic.

Table 1:

Number	Parameter	Iodine-Glycerol	Lactophenol Cotton Blue
1	contrast	poor	better
2	staining	good	poor
3	Uniform staining	excellent	good
4	resolution	good	good
5	Degree of transparency	more	less
6	Visual clarity	better	good
7	Demonstration of Morphology	better	good
8	Artefacts	less	more

Table 2:

	sensitivity	specificity	Positive predictive value	Negative predictive value
Glycerol iodine wet mount	60.5%	97.8%	91.2%	92.6%
Lactophenol cotton blue	55.7%	95.1%	82.2%	88.8%

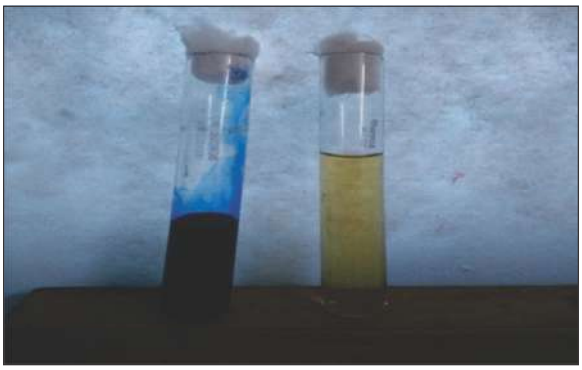

Fig. 1: Lactophenol Cotton Blue and Iodine-Glycerol stains

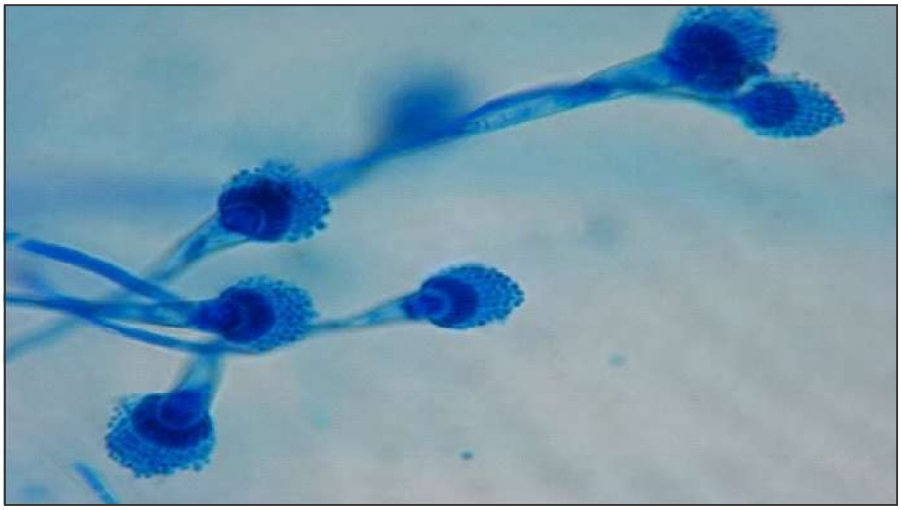

Fig. 2: LPCB of aspergillus flavus

Fig. 3: Iodine-Glycerol mount of Microsporumgypseum

Conclusion

Iodine-glycerol preparation was found to be a better technique for identification fungal isolates which may be employed as a non-hazardous and safer alternative to LPCB for fungal identification.

Acknowledgement

if any: Nil

Source (s) of support in the form of grants, equipment, drugs: Nil.

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.3

Prevalance of Metallo Beta Lactamase Producing Pseudomonas Species in Clinical Specimens from S.S.G Hospital, Vadodara

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Received on 09.12.2018, **Accepted on** 03.01.2019

Abstract

Background: Pseudomonas species are the commonest pathogens causing nosocomial infections. *Pseudomonas* is basically resistant to many antibiotics and they are known to produce Extended Spectrum Beta Lactamase and Metalo beta lactamase.

AIM: To detect Metalo beta lactamase producing *Pseudomonas* spp. from clinical samples in tertiary care hospital.

Material and Methods: The study was agreed over a period of 6 months from January 2015 to June 2015. A total non repetitive of 329 *Pseudomonas* spp were isolated from unusual clinical samples like blood, pus and wound swabs, urine, body fluids, sputum, endo tracheal tube and secretions from the patients attending the hospital. Antimicrobial susceptibility test of all the isolates was performed by the disc-diffusion (Kirby Bauer disc diffusion method) according to CLSIs guidelines. All imipenem resistant isolates were tested for MBL production by Imipenem - EDTA double- disc synergy test (DDST) and Imipenem- EDTA combined disc test (CDT).

Result: Of 329 samples, majority of the *Pseudomonas spp* were isolated from Pus/Wound 173 (52.58%) followed by Blood 103 (31.31%). The isolation rate was highest from pediatrics wards 112 (34.04%) and surgical wards 112 (34.04%). Total 329 samples, 24 (7.29%) isolates were MBL producer by CDST and DDST. Majority of the MBL producing *Pseudomonas spp.* were isolated from Pus/Wound 11 (45.83%) followed by Urine 10 (41.66%). The isolation rate was highest from Surgical wards 9 (37.5%) followed by the Pediatric wards 7 (29.16%).

Conclusion: Detetion of Metallo beta lactamase isolates of *Pseudomonas spp.* will help to implement rational use of antibiotics and strictly adhere to the concept of "reserve drugs" are important to identify because it poses therapeutic problems and serious concern for infection control management.

Keywords: Metalo Beta Lactamase (MBL); *Pseudomonas* Species; Nosocomial Infections.

How to cite this article:

Jignasha Tadvi, Rachna Bhavasar, Hiral Patel et al., Prevalance of Metallo Beta Lactamase Producing Pseudomonas Species in Clinical Specimens from S.S.G Hospital, Vadodara. J Microbiol Relat Res. 2019;5(1):13-19.

Introduction

Pseudomonas species are the common pathogens causing nosocomial infections [1,2]. Infections caused by *Pseudomonas* are either exogenous or endogenous origin, depending on several factors such as use of immunosuppressant agents, injudious use of antimicrobial agents, prolonged surgical procedures and inadequate instrumentations. In recent years due to liberal and empirical use of antibiotics, non fermentative gram negative bacilli have emerged as an important health care associate pathogen. They have been incriminated in infections such as septicemia, pneumonia, urinary tract

infection and surgical site infection. *Pseudomonas* is essentially resistant to many antibiotics and they are known to produce extended spectrum beta lactamase and metallo-beta lactamase. Acquired drug resistance is frequent in nosocomial isolates of *Pseudomonas* spp [1,2]. Acquired Metallo β-Lactamase (MBL) in *Pseudomonas* spp. have recently emerged as one of the most troublesome resistance mechanism because of their capability to hydrolyze all beta-lactam antibiotic including penicillins, cephalosporins and carbapenams, with the exception of Aztreonam [1,2,3,4,5]. Now a days resistance to Aztreonam producing Metallo β-Lactamase (MBL) is also revealed. Currently, there are no recommendations available from CLSI (Clinical and Laboratory Standard Institute) for the detection of MBL. Several phenotypic methods are available for MBL detection. All these methods are based on the ability of metal chelators, such as EDTA and THIOI compounds to inhibit the activity of MBL. In present study, two phenotypic methods were used for the detection of MBL producing *Pseudomonas* species which includes the Imipenem-EDTA combined disc synergy test (CDST) and Imipenem- EDTA double- disc synergy test (DDST) [3,4,5,6].

Aim and Objectives

Aim

- To determine MBL producing *Pseudomonas* spp. from clinical isolates in a tertiary care hospital setting.

Objectives

- To isolate and identify *Pseudomonas* from various clinical specimens (Blood, Body fluids, Sputum, Throat swab etc.)
- To determine antibiotic sensitivity of the isolates to various antibiotics by Kirby-Bauer disc diffusion method
- To screen for MBL producing isolates by detecting resistance to Imipenem (IPM).
- To confirm MBL production in MBL screen test positive by:

a. Imipenem EDTA combined disc synergy test

b. Imipenem EDTA double disc synergy test

- To study sensitivity and resistance pattern among isolates of *Pseudomonas* species from patients admitted in hospital.

Materials And Methods

Study Design: Cross- sectional

Study Setting: Department of Microbiology

Study Subject: The study was carried out over a period of 6 months from January 2015 to June 2015. A total non repetitive of 329 Pseudomonas spp were isolated from different clinical samples like blood, pus and wound swabs, urine, body fluids, sputum, endo tracheal tube and secretions from the patients attending the hospital. Antimicrobial susceptibility test of all the isolates was performed by the disc-diffusion (Kirby Baur disc diffusion method) according to CLSIs guidelines. All imipenem resistant isolates were tested for MBL production by Imipenem - EDTA double- disc synergy test (DDST) and Imipenem- EDTA combined disc test (CDT).

Phenotypic method for detection of Metallo- ß-Lactamases: *[7,8,9,10]*

Preparation of 0.5 M EDTA Solution

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA.$2H_2O$ in 1,000 ml of distilled water. The pH was adjusted to 8.0 by using NaOH and was sterilized by autoclaving. The solution has to be stored at -20^0C.

Combined disk test (CDT): [11,12]

The strains resistant to carbapenems were screened for MBL by CDT. Test was done for detection of metallo- ß- Lactamases in the imipenem resistant isolates. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. 10 µg imipenem disk and IMP (10 µg) + 5µl- 0.5 M EDTA (750 µg) was placed on the agar.. An increase of 7mm or more in zone diameter in the presence of EDTA compared to those with IMP, tested alone was considered to be a positive test for the presence of an MBL.

Double disk synergy (DST) test: [7,8,13,14,15,16]

Test was done for detection of metallo- ß-Lactamases in the imipenem resistant isolates. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. A 10 µg imipenem disk was placed on the agar. A blank disk (6 mm in diameter, Whatmann filter paper no.

1) was kept on the inner surface of the lid of the MHA plate and 10 µl of 0.5 M EDTA is added to it. This EDTA disk was then transferred to the surface of the agar and was kept 10 mm edge-to-edge apart from the imipenem disk. After incubating overnight at 37^0C, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production.

Observation and Results

In this study, majority of *Pseudomonas spp.* were isolated from the age group of 1 to 10 years 91 (27.66%) and 21 to 30 years 65 (19.76%). *Pseudomonas spp* were isolated from male patients 219 (66.57%) as compared to female patients 110 (33.43%) (Table 1).

As can be seen from Table 2, majority of the *Pseudomonas spp.* were isolated from the wound swabs and pus samples 173 (52.58%) followed by the Blood 103 (31.31%). The isolation rate of *Pseudomonas spp.* was highest from the Surgical wards 112 (34.04%) and pediatric wards 112 (34.04%).

In the present study, of the 329 *Pseudomonas spp.* isolates, 24 (7.29%) isolates were MBL producer by CDST and DDST. While 305 (92.17%) isolates were Non- MBL producers (Table 3).

Table 1: Age and Sex distribution of isolated *Pseudomonas spp.* (n=329)

Age in Years	Male	Female	Total
< 1	11	3	14 (4.26%)
1 to 10	53	38	91 (27.66%)
11 to 20	24	12	36 (10.94%)
21 to 30	34	31	65 (19.76%)
31 to 40	28	6	34 (10.33%)
41 to 50	38	5	43 (13.07%)
51 to 60	12	9	21 (6.38%)
61 to 70	17	5	22 (6.69%)
71 to 80	2	1	3 (0.91%)
Total	219 (66.57%)	110 (33.43%)	329

Table 2: Isolation of *Pseudomonas* spp. from various clinical wards and various clinical samples.

Clinical Samples	Blood	Body fluid	Pus/ Wound	Sputum	ET/ TT	Urine	Total
Surgical	0	2	90	1	16	3	112 (34.04%)
Medical	0	0	0	4	1	1	6 (1.82%)
Pediatrics	102	0	0	0	0	10	112 (34.04%)
Orthopedic	0	0	19	0	0	0	19 (5.77%)
Obs & Gynec	0	0	2	0	0	2	4 (1.22%)
Burns	0	0	60	0	0	0	60 (18.24%)
ENT/ Eye	1	0	2	0	0	1	4 (1.22%)
Others (Ward 22,23)	0	7	0	5	0	0	12 (3.65%)
Total	103 (31.31%)	9 (2.73%)	173 (52.58%)	10 (3.04%)	17 (5.17%)	17 (5.17%)	329

Table 3: Prevalence of MBL producer and MBL non-producer of *Pseudomonas spp.*

Isolates	MBL producer N (%)	MBL non producer N (%)	Total N (%)
Pseudomonas spp	24 (7.29%)	305 (92.17%)	329 (100%)

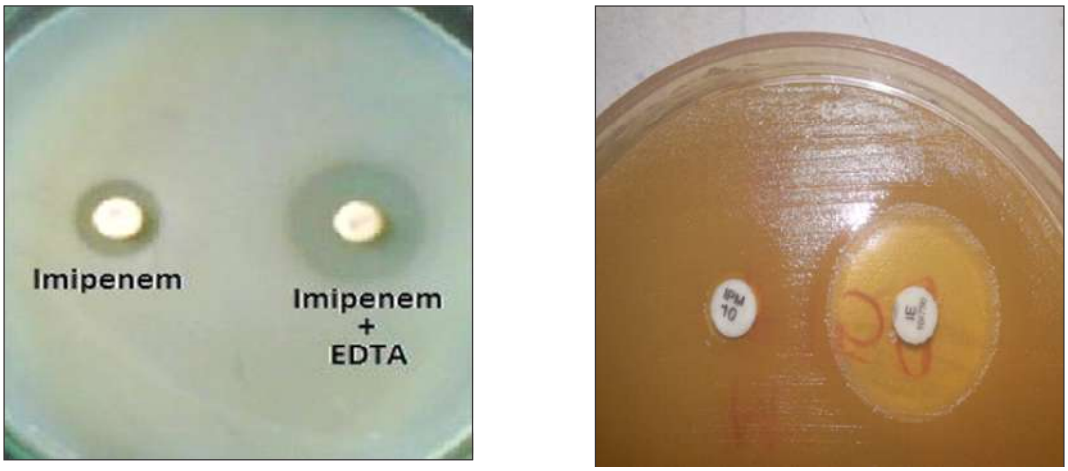

Fig 1: Combined Disk Test (CDT): positive strain shows a ≥ 7mm zone around the Imipenem+EDTA disk.

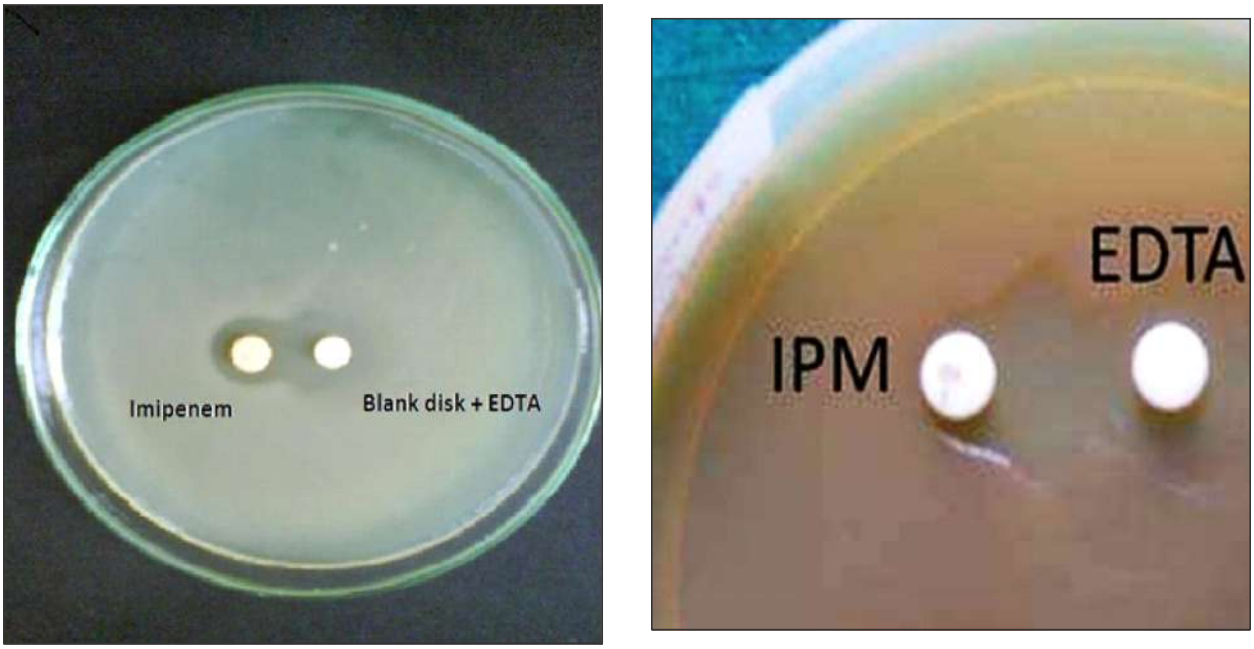

Fig 2: Double Disk Synergy Test (DDST)/ EDTA Disk Synergy Test: Positive strain shows a synergistic zone of inhibition between Imipenem and EDTA disc.

Table 4: Prevalence of MBL producing *Pseudomonas spp.* Fromvarious clinical samples and various clinical wards.

Clinical Samples	Blood	Body fluid	Pus/ Wound	Sputum	ET/ TT	Urine	Total
Surgical	0	0	7	0	1	1	9 (37.5%)
Medical	0	0	0	0	0	0	0 (0%)
Pediatrics	1	0	0	0	0	6	7 (29.16%)
Orthopedic	0	0	1	0	0	0	1 (4.17%)
Obs & Gynec	0	0	0	0	0	2	2 (8.33%)
Burns	0	0	3	0	0	0	3 (12.5%)
ENT/ Eye	0	0	0	0	0	1	1 (4.17%)
Others (Ward 22,23)	0	0	0	1	0	0	1 (4.17%)
Total	1 (4.17%)	0 (0%)	11 (45.83%)	1 (4.17%)	1 (4.17%)	10 (41.66%)	24

As can be seen from Table 4, majority of the MBL producing *Pseudomonas spp.* were isolated from Pus and wound swabs 11 (45.83%) followed by urine samples 10 (41.66%). The isolation rate was highest from surgical wards 9 (37.5%) followed by the pediatric wards 7 (29.16%).

Table 5: Antibiogram pattern of isolates of Pseudomonas spp. to different antibiotics are as follow:

Name of Drugs	Sensitive	Resistance
Piperacillin	278 (84.50%)	51 (15.5%)
Piperacillin + Tazobactam (100µg/ 10 µg)	312 (94.83%)	17 (5.17%)
Amikacin (30 µg)	195 (59.27%)	134 (40.73%)
Cefoperazone (75 µg)	282 (85.71%)	47 (14.29%)
Levofloxacin (5 µg)	278 (84.50%)	51 (15.5%)
Ceftazidime (30 µg)	260 (79.03%)	69 (20.97%)
Gentamicin (10 µg)	197 (59.88%)	132 (40.12%)
Imipenem (10 µg)	305 (92.71%)	24 (7.29%)

All Imipenem-resistant strains in our study showed high resistance to other antibiotics as well**.** High resistance was also observed to Amikacin 134 (40.73%), Gentamicin 132 (40.12%) and also Ceftazidime 69 (20.97%) and 51 (15.5%) isolates were also resistant to Piperacillin and Levofloxacin (Table 5).

Discussion

Pseudomonas spp. are the most frequent nosocomial pathogen and the infections due to these are often difficult to treat because of antibiotic resistance. Acquired drug resistance is frequent in nosocomial isolates of *Pseudomonas spp*. Acquired Metallo-β-lactamases (MBL) in *Pseudomonas spp.* have recently emerged as one of the most worrisome resistance mechanism because of their capacity

to hydrolyze all beta-lactam antibiotics including penicillins, cephalosporins and carbapenems, with the exception of aztreonam. For many years, these MBL producing isolates were restricted to Japan, but now it has disseminated worldwide. [8] In India, MBL producing *P.aeruginosa* was first reported in 2002. [8] *Pseudomonas spp*., a virulent opportunistic pathogens which is one of the major causes of hospital acquired infection has the unique ability to infect all body systems. [17] It almost exclusively infects hospitalized patients with lowered host resistance and is the most frequent pathogens isolated from nosocomial infections in ICU.

In the present study, total 329 isolates from *Pseudomonas* spp. from different clinical samples were studied for their susceptibility or resistance to the antibiotics by (Kirby Baeur disc diffusion method) according to CLSIs guidelines.

Isolation of Pseudomonas spp from various clinical wards and various clinical samples.

In present study isolation of *P.seudomonas spp*. was found to be maximum from wound swabs and pus cultures 173 (52.58%) followed by blood cultures 103 (31.31%). Findings of Horieh Saderi et al. [6] (89.85%), Bashir et al. [9] (46.3%), D.E. Premalatha et al. [18] (58%) and B. Anuradha et al. [19] (39.39%) have also reported a maximum isolation from wound swabs and pus samples which corroborates well with our study. A review of surveillance data collected by the CDC National Nosocomial Infections Surveillance System from 1986 to 1998 shows that P. aeruginosa was identified as the fifth most frequently isolated nosocomial pathogen, accounting for 9% of all hospital-acquired infections in the United States. P. aeruginosa was also the second leading cause of Nosocomial pneumonia (14 to 16%), third most common cause of urinary tract infections (7 to 11%), fourth most frequently isolated pathogen in surgical site infections (8%), and seventh leading contributor to bloodstream infections (2 to 6%).

The isolation of *Pseudomonas spp*. in our study was maximum from the Surgical wards 112 (34.04%) and pediatric wards 112 (34.04%). Rajat Rakesh et al. [20] have stated a similar finding 48% from surgical wards followed by Pediatric wards 23%.

In present study, 7.29% isolates of *P.seudomonas* spp. were found to be MBL producers phenotypically. Most of the authors [6,8,21] have mentioned similar figures. The occurrence of an MBL-positive isolates in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management [6]. In our study majority of the MBL producer Pseudomonas spp. were isolated from pus and wound samples 11 (45.83%) followed by the Urine samples 10 (41.66%). Bashir et al. [9] reported that the predominant source of MBL positive strains was urinary tract (27.3%) followed by wound swabs and pus (27.3%) which correlated with our study. Anil Rajput et al [22] have reported 42.9% MBL producing isolates from Wound swabs and pus samples followed by urine samples 21.4%.

Table 6: Prevalence of MBL producers among *Pseudomonas spp.*

Years of study	Place of study	Authors	MBL Producers
2005	Chennai	Hemalatha et al. [1]	87.50%
2008	Mumbai	Varaiya et al. [3]	20.80%
2008	Pakistan	S Irfan et al. [23]	100%
2008	Iran	Horieh Saderi et al. [6]	53.20%
2009	Puducherry	Noyel et al. [8]	50.00%
2010	Mumbai	Anuradha S De et al. [24]	28.57%
2011	Kashmir	Bashir et al. [9]	11.66%
2011	Tamil Nadu	John and Balagurunathan [25]	14.80%
2011	Ahmadabad	Anil Rajput [22]	12.00%
2011	Pondicherry	Umadevi S et al. [26]	74.50%
2012	Maharashtra	Simit H kumar [27]	32.04%
2013	Kolkata	Rit K et al. [21]	41%
Present study	Vadodara		7.29%

Antibiotic resistance pattern of MBL producing Pseudomonas aeruginosa isolates

All Imipenem-resistant strains in our study showed high resistance to other antibiotics as well. High resistance was also observed to Amikacin 134 (40.73%), Gentamicin 132 (40.12%) and also Ceftazidime 69 (20.97%). 51 (15.5%) isolates were also resistant to Piperacillin and Levofloxacin. This high level of resistance to Aztreonam is very alarming because it is the drug of choice for MBL producing *P.seudomonas* aeruginosa. John and Balagurunathan [25] reported 56.7% resistance to Amikacin, 100% resistance to Piperacillin, Gentamicin and Ciprofloxacin. Kumar SH et al. [27] reported that all MBL –positive isolates were resistant all antibiotics with only 6.06% of the isolates showing susceptibility of Piperacillin/ Tazobactum. Irfan et al. [23] found resistance to antibiotics including third generation cephalosporin, Aminoglycoside and Quinolone. Bashir et al. [9] in his study found that the MBL producers were 100% resistant to ceftazidime, gentamicin and Tobramycin but 100% sensitive to polymyxin B. Rit et al. [21] stated that MBL producing isolates were multi drug resistant except for Colistin (100%) and for Polymyxin B (90%).

Metallo-beta-lactamase enzyme is an emerging threat and cause of concern for physician. The metal ion active site appears to decrease their susceptibility to beta lactamase inhibitors and enable them to hydrolyze broad spectrum including carbapenems. The Metallo-beta-lactamase is plasmid mediated, so the resistance can be spread among hospital pathogen and will cause problems in treating infections.

The prevalence of detect Metallo-beta-lactamase producing *P.seudomonas* spp. in our setup was 7.29%.

Conclusion

MBL (metallo-β-lactamase) positive isolates of *P.seudomonas* spp. are important to identify because it poses not only therapeutic problem, but also a serious concern for infection control management. There is also a need to emphasize on the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

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STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS

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

1. Place of Publication	:	Delhi
2. Periodicity of Publication	:	Quarterly
3. Printer's Name	:	**Asharfi Lal**
Nationality	:	Indian
Address	:	3/258-259, Trilok Puri, Delhi-91
4. Publisher's Name	:	**Asharfi Lal**
Nationality	:	Indian
Address	:	3/258-259, Trilok Puri, Delhi-91
5 Editor's Name	:	**Asharfi Lal** (Editor-in-Chief)
Nationality	:	Indian
Address	:	3/258-259, Trilok Puri, Delhi-91
6. Name & Address of Individuals	:	**Asharfi Lal**
who own the newspaper and particulars of	:	3/258-259, Trilok Puri, Delhi-91
shareholders holding more than one per cent of the total capital		

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.4

Invasive *Group B Streptococcal* Infection in Non-Pregnant Adult Patients in A Tertiary Care Centre

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Received on 07.12.2018, **Accepted on** 04.02.2019

Abstract

Introduction: Group B streptococcus (GBS) is an important cause of illness in newborns, pregnant women as well as in non-pregnant elderly patients particularly those with significant underlying diseases. The study was carried out to determine the clinical spectrum, antibiogram and outcome of *Group B Streptococcus infection* as it can cause invasive infections in non-pregnant adults that can lead to substantial morbidity and mortality in them.

Materials and Methods: A retrospective observational chart based study was conducted in a tertiary care centre for a period of one year (June 2016 to May 2017). Multisite samples like pus, wound swabs, blood, body fluids and urine samples were collected from male and female adult patients (adults were defined as >18 years old) with Group B streptococcus infections. The samples were processed for culture and sensitivity aerobically according to the standard operating guidelines. Statistical analysis of the data was performed using SPSS v.21 by frequency, percentage, Fisher's exact test and a p value less than 0.5 was considered as significant.

Results: In 156 cases of GBS infections among non-pregnant adults, the prevalence was higher in the age group of 50-64 years (37.8%) with female to male ratio of 1.1:1. The most frequent clinical presentations were urinary tract infections (65.4%) with a significant female gender predominance (p value <0.0001). All tested isolates were susceptible to Ampicillin, Cephalosporins, Penicillin and Vancomycin.

Conclusion: Elderly people with underlying diseases are more prone to GBS infections. Therefore, evaluation of risk factors and development of an antibiotic policy is useful for the successful treatment of GBS infections.

Keywords: Noninvasive; Non-Pregnant; *Streptococcus Agalactiae; Group B Streptococcus*.

How to cite this article:

Pampi Majumder & Thomas S. Kuruvilla. Invasive *Group B Streptococcal* Infection in Non-Pregnant Adult Patients in A Tertiary Care Centre. J Microbiol Relat Res. 2019;5(1):21-26.

Introduction

Group B streptococcus (GBS) is a normal commensal of the gastrointestinal and genitourinary tracts, but it's also an important cause of illness in newborns, pregnant women, and non-pregnant elderly patients particularly those with significant underlying diseases like diabetes, neurological impairment, cirrhosis and increase risk for invasive *Group B Streptococcal* (GBS) disease. Common presentations are skin, soft-tissue, and osteo-articular infections, pneumonia, and urosepsis etc. [1] Though meningitis, *Streptococcal* toxic shock syndrome and endocarditis are less common, its associated with serious morbidity and mortality. The main manifestations of GBS in infancy are bloodstream infections, with or without pneumonia. Incidence

of invasive GBS infections has increased two to four-fold in non-pregnant adults over the last 2 decades, with rates ranging from 4.1 to 7.2 cases per 100,000 non pregnant adults [1]. The severity of GBS infection increases with age; the mean age of non- pregnant adults with invasive GBS disease is about 60 years, and the associated mortality rate is close to 25% [1]. On an average 1(5%) out of every 20 non-pregnant adults with invasive Group B Streptococcal infections die [1]. Between 15% and 35% of pregnant women are asymptomatic carriers of GBS, and in the early 1990's 0.2 to 0.8% of neonates had GBS bacteremia [2]. GBS is regarded as uniformly susceptible to penicillin, but recent reports have highlighted the emergence of strains resistant to erythromycin and clindamycin [3]. The resistant strains raise the importance of the use of erythromycin and clindamycin for the prophylaxis or treatment of GBS infections in patients allergic to beta-lactams [3].

Moreover, increasing antimicrobial resistance has implications for GBS disease treatment and intrapartum prophylaxis among penicillin intolerant patients. This present work deals with the clinical spectrum and antibiogram of *Group B Streptococcus* that can help in better approach towards empirical treatment and outcome of these patients.

Materials and Methods

This retrospective observational chart based study was carried out for a period of one year from June 2016 to May 2017, in the Department of Microbiology, in a tertiary care centre, after ethical clearance. Clinical data, epidemiological and demographic profile of each case including comorbidities and outcomes were abstracted by chart review. Multisite samples like pus, wound swabs, blood, body fluids and urine samples were collected from male and female adult patients (adults were defined as anyone >18 years old) with *Group B streptococcus* infections, which were received in the laboratory from the ailing patient and outpatient facilities, were included in the study. For patients who had more than one GBS culture positive during the study period, only the first episode of GBS infection was analyzed. Vaginal samples, samples from pregnant women and samples from neonates were excluded from this study. The samples were cultured aerobically on 5% Sheep blood agar and MacConkey agar and *Group B Streptococcus* identification was based on the following criteria: a narrow zone of beta-hemolytic colonies on 5% sheep blood agar plate (Fig. 1), Gram-positive cocci in pairs or short chains on Gram's staining, a negative-catalase reaction, positive hippurate hydrolysis test, positive reaction with Christie, Atkins, Munch-Peterson (CAMP) (Fig. 2) and a bacitracin differential disk resistance pattern. Antimicrobial susceptibility testing was done on Mueller Hinton agar supplemented with 5% sheep blood by Kirby-Bauer disc diffusion method according to the updated CLSI guidelines. Statistical analysis of the data was performed using SPSS v. 21 by frequency, percentage and Fisher's exact test.

Fig. 1: *Group B Streptococcus* showing beta hemolysis on Sheep Blood Agar

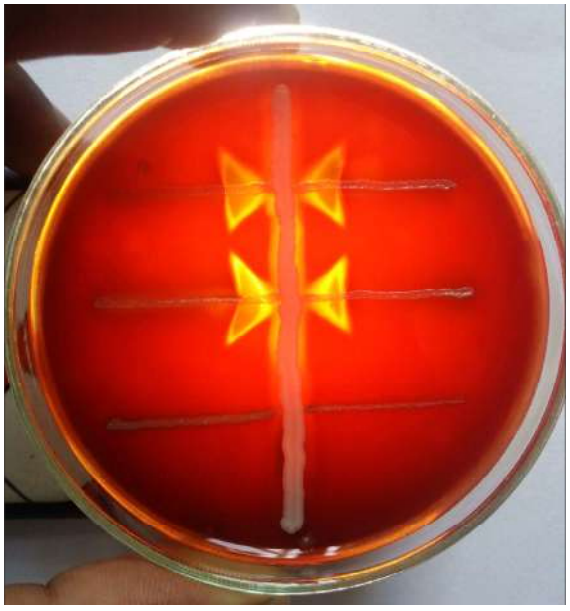


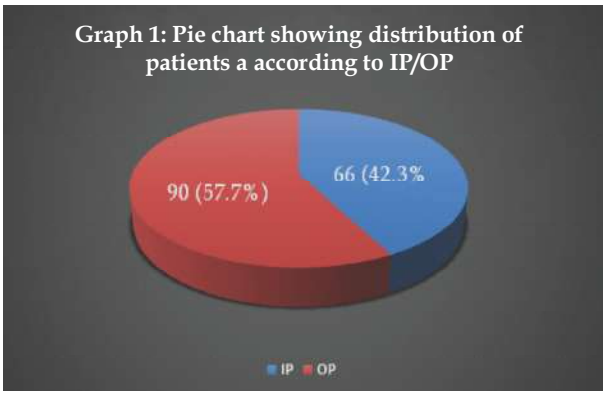
Fig. 2: *Group B Streptococcus* showing positive CAMP test on Sheep Blood Agar

Results

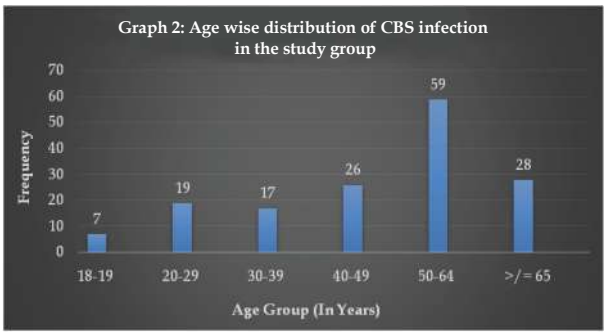
There were 156 cases of GBS disease among non-pregnant adults. Most of the cases in our study were outdoor cases ie. 57.7% from clinics and hospitals around Mangalore (Graph 1). Of these, 83 (53.2%) of the non-pregnant cases occurred in women and 73 (46.8%) cases in men. The prevalence of GBS was higher in the age group of 50-64 years (37.8%), followed by ≥ 65 years (17.9%) with female and male ratio of 1.1:1 (Graph 2). A review of the clinical records of the 156 adult cases revealed that the most frequent clinical presentations was urinary tract infections (65.4%) followed by wound and soft-tissue infections (32.7%) (Table 1). Out of 156 GBS cases, polymicrobial infections occurred in 10 (6.4%) cases of GBS i.e 6 urinary tract infections and 4 soft tissue infections. The most common additional pathogens were *Staphylococcus aureus, Coagulase-negative Staphylococcus and Escherichia coli*. Among 66 (42.3%) admitted patients 58 (87.9%) of the non pregnant patients had > 1 underlying medical conditions or predisposing factors. These were diabetes, surgical intervention, cancer and hospitalization in decreasing order of frequency (Table 2). Among the 17 (29.3%) non pregnant cases associated with nosocomial transmission, 11 (64.7%) patients had undergone surgery before developing the GBS infection and the other 6 (35.3%) cases had been hospitalized for more than 48 hours before the first positive culture. In the current study 2 cases had diabetes mellitus with hypothyroidism and baker's cyst.

An HIV positive case was reported in our study. All tested isolates were susceptible to ampicillin, cephalosporins, penicillin and vancomycin. Resistance to erythromycin and clindamycin were common being 52 (33.3%) and 31 (19.9%) respectively. The prevalence of levofloxacin resistance was low 2 (1.3%). None of the Group B Streptococcus isolates showed resistance to ciprofloxacin. (Table 3)

Out of 156 non pregnant adult patients 7 (4.5%) died while hospitalized and 3 (42.8%) deaths were attributed to GBS infection. The deaths included 2 men (an 80 years old who died of acute kidney injury and sepsis, and a 58-years old with dilated cardiomyopathy who presented with abdominal cellulitis and died of sepsis). A 64-year-old woman died of corpulmonale, multi organ dysfunction syndrome and sepsis. The mean interval between positive culture and death was 4 days. 35 (53%) of the 66 hospitalized patients (in patients) were still hospitalized 10 days after their diagnosis with GBS disease. The 90 outpatients were treated on an outdoor basis and showed significant recovery on follow up without any complications.



Graph 1: Pie chart showing distribution of patients according to Inpatient & Outpatient data



Graph 2: Age wise distribution of GBS infection in the study group

Table 1: Clinical spectrum of GBS in the study group

Clinical presentation	Male (%)	Female (%)	Total (%)	p value
Urinary tract infection	34(33.33%)	68 (66.67%)	102 (65.4%)	p = 0.000, <0.0001 (Fisher's exact test)
Bone & Soft tissue infections	31(75.60%)	10 (24.40%)	41(26.3%)	
Wound infection	6(60%)	4 (40%)	10 (6.4%)	
Primary bacteremia	2 (66.67%)	1 (33.33%)	3 (1.9%)	
Total	73 (46.79%)	83 (53.20%)	156 (100%)	

*p value <0.05 is significant

Table 2: Underlying conditions of non pregnant adult patients with GBS infection.

Co-morbidity	Male (%)	Female (%)	Total (%)
Diabetes mellitus	16 (57.14%)	12 (42.86%)	28 (42.4%)
Surgery	7 (63.64%)	4 (36.36%)	11 (16.7%)
Carcinoma	7 (70%)	3 (30%)	10 (15.2%)
Hospitalized for ≥48 hours before first positive culture report	4 (66.67%)	2 (33.33%)	6 (3.8%)
Renal disease	3 (50%)	3 (50%)	6 (3.8%)
Cardiac disease	1 (25%)	3 (75%)	4 (2.6%)
Multi organ dysfunction syndrome	3 (100%)	-	3 (1.9%)
Liver disease	1 (50%)	1 (50%)	2 (1.3%)
Vascular disease	1 (50%)	1 (50%)	2 (1.3%)
Previous trauma to the infected site	-	1 (100%)	1 (0.6%)
HIV positive case	1 (50%)	-	1 (0.6%)
Unknown / not specified	7 (58.33%)	5 (41.67%)	12 (7.7%)

Table 3: Susceptibility and resistance pattern (in percentage) of various antibiotics

Antibiotics	Sensitive (%)	Resistant (%)
Penicillin	100	-
Ampicillin	100	-
Cefazolin	100	-
Ceftriaxone	100	-
Ciprofloxacin	100	-
Levofloxacin	98.7	1.3
Erythromycin	66.7	33.3
Clindamycin	80.1	19.9
Vancomycin	100	-
Linezolid	100`	-

Discussion

Group B Streptococcus (GBS) also known as *Streptococcus agalactiae,* belongs to Group B of the Rebecca Lancefield classification of *Streptococci*. It is a Gram-positive cocci which is beta-haemolytic on blood agar, catalase negative and a facultative anaerobe. GBS was considered to be a veterinary pathogen as it caused bovine mastitis in dairy cows [4]. Its surrounded by a polysaccharide capsule and further subclassified into 10 serotypes (Ia, Ib, II–IX). Various virulence factors play role in the pathogenesis of GBS infection, but the most important are the capsular polysaccharide which is rich in sialic acid, and a pore-forming toxin, β-hemolysin. Capsular polysaccharide helps GBS to evade host defense mechanisms by interfering with phagocytosis [5,6].

GBS is best known to cause postpartum infection and neonatal sepsis, but it has become an emerging cause of invasive infection in non-pregnant adults also. This multisite population based analysis reveals that all age groups are subjected to GBS infection. GBS is more common to cause bimodal age distribution affecting young and middle aged healthy women secondary to obstetrical manipulation and also elderly persons with pre-existing illness [4].

There is no sexual predilection of *Group B Streptococcal* infection among non-pregnant adults, [4] but in our study the prevalence of GBS was slightly higher in females (53.2%) with a male to female ratio 1.1:1 which corresponds with an active surveillance study by Tyrrell G J et al. [7] Previous studies by Lee N Y et al. [8] and Tazi A et al. [9] demonstrated high male preponderance of invasive GBS infections in adults.

In the current study the incidence was higher in older patients of (50-64) years age group followed by ≥ 65 years which are similar to previous reports [9,10]. GBS disease among non-pregnant adults are not well understood as it is said that underlying comorbidities resulting in defective phagocytic function and altered integrity of anatomical barriers with age promote GBS invasion [11]. In support of the previous findings [2], diabetes mellitus (42.4%) had a higher prevalence followed by surgical procedures (16.7%) and carcinoma (15.2%) in the present study.

Many studies have described GBS as a causative agent of skin and soft tissue infections, respiratory infections, sepsis, meningitis, endophthalmitis and urinary tract infections especially in women [2,10,11]. In the current study the most common presenting symptom was urinary tract infection (65.4%) followed by skin and soft tissue infection (32.7%) and bacteremia (1.9%) without any focus which is in agreement with previous reports [12]. Urinary tract infection was more common in female 68 (66.67%) whereas skin and soft tissue infection was more common in male patients 31 (75.60%) in our study which was found to be highly significant statistically (p value <0.0001). As GBS can colonize

in women urethra the prevalence of UTI was higher in women 66.7% (68/102) compared to men 33.3% (34/102) in the current study. Study of invasive GBS in non-pregnant adults by Tazi A et al., out of total 401 patients revealed most GBS strains were primarily isolated from cases of bacteremia without any focus (43.4%) followed by bone and joint infections (18.7%), skin and soft tissue infections (12%), endocarditis (10.5%), meningitis (5.2%), respiratory tract infections (4%), peritonitis (3.2%) and urinary tract infections (3%) [9].

In our study we did not report any GBS causing meningitis, pneumonia, endocarditis or endophthalmitis cases. In adults though GBS causes low bacteremia without any focus or obvious source but sustained bacteremia may result from infected central venous catheter or endocarditis [4]. Skin and soft tissue infections by *Streptococci agalactiae* may result in fatal prognosis ranging from mild cutaneous ulcers, cellulitis to osteomyelitis and limb amputation which lead to substantial mortality and morbidity in non-pregnant adults [8]. In our study the most common bone and joint infection was osteomyelitis followed by osteoarthritis and knee effusion.

Group B Streptococci causes significant mortality in both neonates and adults but mortality rate is higher in elderly patients with comorbid medical conditions [4]. In the current study the overall mortality rate was 4.5% (7/156) where 3 (42.8%) deaths were attributed to GBS infection. The deaths included 2 men (an 80 years old who died of acute kidney injury and sepsis, and a 58-years old with dilated cardiomyopathy who presented with abdominal cellulitis and died of sepsis) and a 64 years old woman who died of corpulmonale, multi organ dysfunction syndrome and sepsis. The mean interval between positive culture and death was 4 days.

Penicillin is the drug of choice for both prophylaxis and treatment of GBS infections and so far, no resistance has been reported [13]. However, macrolides are the second-line agents and recommended for patients with penicillin allergy [14]. In the current study antimicrobial susceptibility of all 156 isolates showed susceptibility to ampicillin, cephalosporins, penicillin, and vancomycin (all being 100% susceptibility) despite increasing antibiotic use which is consistent with other published reports [7,9,15,16].

Correlating with previous studies, [9,16] resistance to erythromycin and clindamycin were common being 52 (33.3%) and 31 (19.9%) in our study. Matsubara K and Yamamoto G reported 2% and 3% erythromycin and clindamycin resistance, respectively in their study of invasive *Group B streptococcal* infections [15]. In a study of prevalence of macrolide resistance in invasive and noninvasive *Group B Streptococcus* isolates, erythromycin resistance was present in 8% of strains, with 4.5% resistance to clindamycin [14]. The most common macrolide resistance mechanisms in *Streptococci* are ribosomal modification by a methylase encoded by an *erm* [17] gene and drug efflux by a membrane-bound protein encoded by a *mef* gene [18]. The prevalence of levofloxacin resistance was low 2 (1.3%) and none of the *Group B Streptococcus* isolates showed resistance to ciprofloxacin and levofloxacin in our study. Further serotyping of GBS isolates and determination of the MIC (Minimum Inhibitory Concentration) value of antimicrobial agents could have helped to target a specific vaccine candidate.

Conclusion

The increasing rates of GBS infections needs an evaluation of risk factors and development of an antibiotic policy to successfully treat GBS infections and minimize its life threatening complications and emergence of resistant strains.

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.5

Aerobic Bacterial Isolates from Diabetic Foot Ulcers and Their Antibiotic Susceptibility Pattern in A Tertiary Care Hospital

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Received on 28.01.2019, **Accepted on** 07.03.2019

Abstract

Background: Diabetes mellitus is a metabolic disorder in which there is increase in the levels of blood glucose because of insulin deficiency. Diabetic foot ulceration and infections are major medical, social, economical problem and it is the leading cause of morbidity and mortality in developing countries like India. The present study is an attempt made to know the aerobic bacteriological profile of diabetic foot ulcers.

Material and methods: A total of 100 patients with diabetic ulcers admitted in surgical wards were studied. Pus was collected using two swabs from each patient one for staining and the other for aerobic culture. The organisms isolated were identified using standard techniques. Antimicrobial susceptibility of the bacterial isolates was done by Kirby-Bauer disc diffusion method.

Result: Polymicrobial etiology was observed in 59% and monomicrobial etiology in 41%. A total of 165 organisms were isolated. Most common isolates were *staphylococcus aureus* 38 (23.03%), followed by *Klebsiella spp* 34 (20.6%), *Pseudomonas aeruginos*a 28 (16.96%), *Escherichia.coli* 26 (15.75%), *Proteus spp* 23 (13.93%), *Enterococcus faecalis* 8 (4.84%), *Citrobacter spp* 4 (2.42%) and *Staphylococcus epidermidis* 4(2.42%). Most sensitive antibiotics were Imipenem, amikacin, ciprofloxacin and Gentamicin.

Conclusion: Culture and sensitivity from the wound plays an important role in prescribing the appropriate antibiotic at the time of admission itself rather than starting empirical treatment. Thus proper antibiotics policy and measures to restrict the indiscriminate use of antibiotics should be taken to minimize the emergence of drug resistant pathogens.

Keywords: Diabetic Foot Ulcers; *Staphylococcus aureus; Klebsiellaspp; Citrobacter Spp*; Polymicrobial etiology.

How to cite this article:
Sangeetha C. Patil, Sumathi S, Surekha Y Asangi et al. Aerobic Bacterial Isolates from Diabetic Foot Ulcers and Their Antibiotic Susceptibility Pattern in A Tertiary Care Hospital. J Microbiol Relat Res. 2019;5(1):27-33.

Introduction

Diabetes mellitus (DM), a very common endocrine disorder with major public health consequences arising from severe damage to numerous end organs. DM affects all populations worldwide and the prevalence of this disease is increasing at a very alarming rate. The International Diabetes Federation (IDF) currently estimates that about 366 million persons in the world have DM, with projections that this will increase to 552 million by 2030 [1]. The Indian diabetic population is expected to increase to 57 million by the year 2025 [2]. At present 31.7 million people are diabetic

in India. Hence, it has been labelled as "The diabetic capital of the world". Diabetes warrants a lot of attention because of its various complications like retinopathy, nephropathy, peripheral neuropathy, cardiovascular disease, peripheral vascular disease (PVD), cerebrovascular accident, hypertension and diabetic foot [3].

The diabetic foot may be defined as a group of syndromes in which neuropathy, ischemia , and infection lead to tissue break down resulting in morbidity and possible amputation [4]. About 15-25% will develop a diabetic foot ulceration (DFU) during their lifetime, Over 50% of these ulcerations will become infected. Re admission rates for DFI patients are approximately 40% and nearly one in six patients die within 1 year of their infection. The presence of infection in a patient with DFU increases the risk of a minor amputation by 50% compared to patients with ulcers which are not infected [5]. The diabetic wounds are mostly infected by pus forming microorganisms like *Enterococcius spp, Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiella spp, Proteus spp* [6].

Aims and Objectives

1. To isolate the pathogenic organisms from diabetic foot ulcers.
2. To determine the antibiotic susceptibility pattern of isolated organisms.

Materials and Methods

The present study was conducted in the Department of Microbiology, Vijayanagar Institute of Medical Sciences, Bellary for duration of one year. A total of one hundred patients with diabetic ulcers admitted in surgical wards were studied.

A proforma was filled for each patient documenting such as age, sex, address and clinical information including chief complaints, duration of symptoms, predisposing factors and any previous history of treatment.

Collection of Sample

Samples were collected in the surgical wards where the dressing was being done. The ulcer was cleaned with sterile normal saline and the surrounding area was cleaned with 70% alcohol. Debris, dead and devitalized tissue overlying the ulcer was removed using a sterile forceps and scissors. Swabs were collected from the depth of the ulcers on the feet of the diabetic patients. From each patient, two swabs were collected. One swab was used for the isolation of aerobic bacteria and the other for preparation of smear for Gram stain. [7]. Debrided necrotic material was also collected [8]. After sample collection, the specimens were processed immediately in the laboratory.

Processing of Sample

Direct microscopic examination: Smear was prepared on clean glass slide, air dried. Gram stain was done for the smear and examined under oil immersion objective for the presence of pus cells, bacteria and fungi, low power and the high power objectives for fungi [9].

Culture

Aerobic culture: The swabs were inoculated on nutrient agar, blood agar and MacConkey agar. All plates were incubated aerobically at 37^0C and evaluated at 24 hours, 48 hours and 72 hours. The organisms isolated were identified using standard techniques, based on the colony morphology, Gram staining of smear from colony and biochemical properties.

Antimicrobial susceptibility of the bacterial isolates to the commonly used antibiotics was done by Kirby-Bauer disc diffusion method [9,10].

The strength of the antibiotics discs used were [11].

Ampicillin 10 µg

Amoxicillin/Clavulanic acid

Augmentin 20 µg/10 µg

Amikacin 30 µg

Gentamicin 10 µg

Ciprofloxacin 5 µg

Ceftriaxone 30 µg

Cefotaxime 30 µg

Imipenem 10 µg

Microbial agents of diabetic foot ulcers

Most of the diabetic foot infections are polymicrobial in nature and mixed organisms are frequently encountered. Spectrum of microorganisms depends mainly on microbial flora of the lower limb, metabolic factors, food hygiene, and the use of antibiotics[2].

Results

Table 1: Age and sex distribution

Age group	Male		female		Total	
	No	%	No	%	No	%
21-30	2	3.08	0	0	2	2
31-40	1	1.54	2	5.72	3	3
41-50	14	21.53	13	37.14	27	27
51-60	27	41.54	10	28.57	37	37
>60	21	32.31	10	28.57	31	31
Total	65	100	35	100	100	100

Out of 100 cases, 65 were males and 35 were females. Among 100 cases, 37 (37%) were of age group 51-60 years, out of 37, 27 (41.54%) were males and 10 (28.57%) were females. 31 (31%) cases were of age group 61 and above. Out of 31, 21 (32.3%) were males and 10 (28.57%) were females. 27 (27%) cases were of age group 41-50 years, 3 (3%) cases were of between 31-40 years, 2 (2%) cases were of age group of 21- 30 years (Table 1).

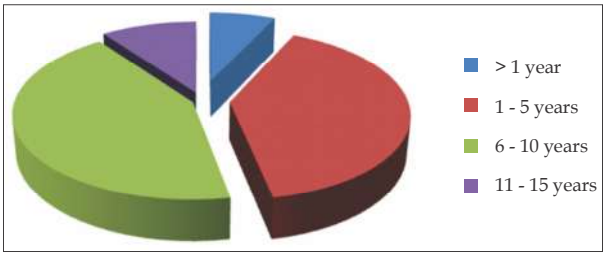

Fig 1: Showing the duration of diabetes mellitus

Above Fig. 1 shows that out of 100 cases. 43 (43%) had diabetes for 6-10 years. 40 (40%) had diabetes for 1-5 years. 10 (10%) had diabetes mellitus for 11-15 years and 7 (7%) were detected diabetic at the time of admission for the treatment of ulcer.

It is observed that most of the patients were suffering from diabetes for more than five years.

Type of Diabetes Mellitus

Out of 100 cases, 2 (2%) cases were insulin dependent diabetes mellitus and 98 (98%) cases were non insulin dependent diabetes mellitus.

Table 2: Duration of diabetic ulcer

Duration in weeks	Number of cases	Percentage
<1 week	5	5
2-4	40	40
5-7	15	15
8-10	30	30
>11 weeks	12	12
Total	100	100

Majority of the patients 40 (40%) were presented with ulcer of 2-4 weeks duration followed by 30 (30%) patients presented with ulcer of 8 -10 weeks duration, 15 (15%) patients presented with ulcer of 5- 7 weeks duration. 12 (12%) patients presented with ulcer of more than 11 weeks and 5(5%) patients had ulcer of less than 1 week (Table 2).

It is observed that, most of the patients presented with ulcer of more than two weeks duration

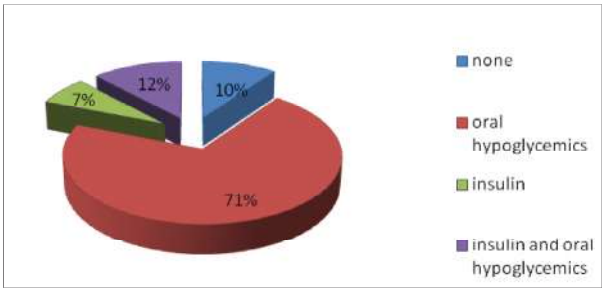

Fig. 2: Showing treatment taken for diabetes mellitus

The above Fig. 2 shows that , prior to admission, 71 (71%) patients were maintained on oral hypoglycaemic agents , 12 (12%) patients were both on insulin and oral hypoglycaemics, 7 (7%) were on insulin therapy and 10 patients were not previously diagnosed as diabetics.

Table 3: showing the different aerobic organisms isolated

Type of organism	Number of organisms	Percentage
Gram positive organisms	50	30.3
Staphylococcus aureus (38)	38	23.03
Staphylococcus epidermidis (4)	4	2.42
Enterococcus faecalis (8)	8	4.84
Gram negative organisms	115	69.6
Pseudomonas aeruginosa (28)	28	16.96
Klebsiella pneumoniae (32)		
Klebsiella oxytoca (2)	34	20.6
E.coli(26)	26	15.75
Proteus mirabilis (21)		
Proteus vulgaris (2)	23	13.93
Citrobacter freundii (3)		
Citrobacter koseri (1)	4	2.42
Total	165	100

Out of 165 organisms isolated, most common isolates were staphylococcus aureus 38 (23.03%), followed by Klebsiella spp 34 (20.6%), Pseudomonas aeruginosa 28 (16.96%), Escherichia. coli 26 (15.75%), Proteus spp 23 (13.93%), Enterococcus fecalis 8 (4.84%), Citrobacter spp 4 (2.42%) and Stapylococcus epidermidis 4 (2.42%) (Table 3).

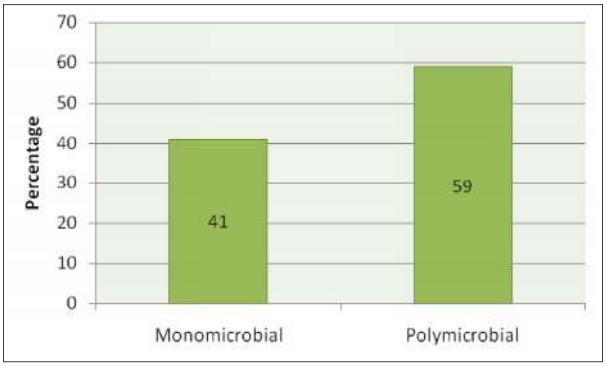

Fig. 3: Showing the distribution of organisms

The above Fig. 3 shows that monomicrobial flora isolated in 41% of cases and polymicrobial flora isolated in 59% of cases.

Table 4: Distribution of organisms in polymicrobial flora

Type of organism	Number of cases	Percentage
Gram positive organisms	2	3.38
Gram negative organisms	24	40.67
Gram positive and Gram negative organisms	26	44.06
≥ 3 organisms	7	11.86
Total	59	100

Majority of the organisms isolated Gram positive and negative oranisms from (44.06%) cases followed by Gram negative organisms in 40.67%, three organisms isolated in 11.86% of cases and Gram positive organisms in 3.38% of cases (Table 4).

to ciprofloxacin, 56 (33.9%) were sensitive to Gentamicin, 55 (33.3%) were sensitive to ceftriaxone, 48 (29%) were sensitive to cephalexin, 43 (26%) were sensitive to amoxycillin and clavulanate, 40 (24.2%) were sensitive to cefotaxime.

From the above antibiogram most sensitive antibiotics were Imipenem, amikacin, ciprofloxacin and Gentamicin.

Discussion

Patients with DM frequently require minor or major amputations of the lower limbs (15–27%), and in more than 50% of cases, infection is the preponderant factor. These more severe diabetic foot infections usually require hospitalization, parenteral antibiotic therapy and surgical procedures [12].

Most of the cases if identified early and treated appropriately initially in the community can be treated effectively with antibiotics at an early stage and in an out-patient setting. But unfortunately because of the late referrals primarily and also alternate medicines, herbal medicines, and poor medical facilities in the far flung and tribal areas, less knowledge regarding diabetes in general and foot ulcers in particular leads to loss of limbs and loss of life in some cases even when they reach a tertiary care hospital [13].

Table 5: Antibiotic susceptibility pattern of isolates

Antibiotics	*Staphylococcus aureus* (n=38)	*Staphylococcus epidermidis* (n=4)	*Enterococcus spp* (n=8)	*Pseudomonas spp* (n=28)	*Klebsiella spp*(n=34)	*E. coli* (n=26)	*Proteus spp* (n =23)	*Citrobacter spp* (n=4)	Total n=165
Amikacin	17 (44.7)	1 (25)	3 (37.4)	14 (50)	19 (55.8)	14 (53.8)	15 (65)	3 (42.9)	86 (52.1)
Amoxycillin and clavulanate	15 (39.4)	3 (75)	5 (62.5)	0 (0)	6 (17.6)	7 (26.9)	7(30.4)	0(0)	43 (26)
Gentamicin	10 (26.3)	1 (25)	2 (25)	9 (32.1)	13 (38.2)	11 (42.3)	8 (38)	1 (33.3)	56 (33.9)
Ciprofloxacin	13 (34.2)	3 (75)	5 (62.5)	11 (39.2)	21 (61.7)	18 (69.2)	11 (52.4)	3 (42.9)	85 (51.5)
Ceftriaxone	12 (31.5)	2 (50)	4 (50)	3 (10.7)	12 (35.2)	12 (46.1)	9 (39.1)	1 (25.3)	55 (33.3)
Cefotaxime	6 (15.7)	2 (50)	1(12.5)	1 (3.5)	9 (26.4)	12 (46.1)	8 (38)	1 (25.3)	40 (24.2)
Imipenem	34 (89.4)	3 (75)	6 (75)	25 (89.2)	30 (88.23)	24 (92)	21 (87)	4 (100)	147 (89)
Cephalexin	11 (28.9)	2 (50)	2 (25)	1 (3.57)	21 (61.7)	4 (15.3)	7 (33.3)	1 (25.3)	48 (29)

The above table 5 shows the antibiotic susceptibility pattern of aerobic organisms isolated in the study. Out of 165 organisms isolated 147 (89%) were sensitive to imipenem, 86 (52.1%) were sensitive to amikacin, 85 (51.5%) were sensitive

Duration of diabetes mellitus

In the present study 53 (53%) of cases were suffering from diabetes for more than 5 years. This finding is in concordance with study done by Sapico et al. [14] 8 (61.54%) were suffering from

diabetes for more than 5 years, and Leela Rani K et al. [7] reported 53.6% of cases were suffering from diabetes mellitus for more than 6 years.

IDDM/NIDDM

In the present study out of 100 cases, 98% were non- insulin dependent diabetes mellitus. This correlated with study done by Ramani et al. (81.34%) [15], Chincholikar et al. (76.19%) [16], Ravishekhar Gadepalli et al. (88.8%) [17], Azizul Hasan et al. (92%) [13], with the predominance of type 2 diabetes mellitus.

Comparison of monomicrobial and polymicrobial flora

In the present study monomicrobial etiology found in 41% of cases and polymicrobial etiology in 59%. Similar findings were observed in study done by Chincholikar et al. [16] for monomincrobial (30.5%) and polymicrobial (69.5%), Kavitha A et al. [3] for monomincrobial (25%) and polymicrobial (85%), Leela Rani et al. [7] for monomincrobial (36%) and polymicrobial (56%), Dushyanth singh et al. [18] for monomincrobial (14.75%) and polymicrobial (85.24%).

Comparison of aerobic organisms isolated

In the present study 165 aerobic organisms isolated. The most predominant organisms isolated were *staphylococcu aureus* 38 (23.03%), followed by *Klebsiella* spp 34 (20.6%), *Pseudomonas aeruginosa* 28 (16.96%), *E.coli* 26 (15.75%), *Proteus* spp 23 (13.93%), *Enterococcus fecalis* 8 (4.84%), *Citrobacter* spp 4 (2.42%) and *Staph epidermidis* 4 (2.42%).

Sapico et al. [14] reported, most predominant organisms isolated were *Proteus spp* (13.3%) followed by *Staphylococcus aureus* (10%), *E.coli* (10%), *Enterobacter spp* (9.9%), *Enterococcus spp* (5%) and each 3.3% by *Staphylococcus epidermidis*, *Streptococcus spp*, *Pseudomonas spp*, *Providendia spp*, *Citrobacter spp*.

Ramani et al. [15] reported, most predominant organisms isolated were *Staphylococcus aureus* (60%) followed by *Klebsiella spp* (20%), *Proteus spp* (19%), *Pseudomonas spp* (19%), *Enterococcus spp* (11%), *Citrobacter spp* (9.2%), *Staphylococcus epidermidis* (4.9%), *E.coli* (2.4%), and each 3.3% by *Streptococcus spp (1.8%) and Enterobacter spp (1.8%).*

Rovan urbancic et al. [18] reported, most predominant organisms isolated as *Staphylococcus aureus* (26.7%), *Staph epidermidis* (9.9%), *Enterococcus spp* (9.5%), *Klebsiella spp* (5.4%), *Enterobacter spp* (3.6%), and *E.Coli* (3.2%).

Ahamed T [19] reported, most predominant organisms isolated were *Staphylococcus aureus* (28%) followed by *Pseudomonas spp* (22%), *Proteus spp* (18%), *Streptococcus spp* (13%), *Enterococcus spp* (11%), *Staphylococcus epidermidis* (7%), *Klebsiella spp* (6%), *E.coli* (6%), *Enterobacter spp* (5.5%), *Morganella spp* (5.5%)

Ravishekhar et al. [17] reported, most predominant organisms isolated were *Pseudomonas spp* (18%), *Staphylococcus aureus* (13.7%), *Proteus spp* (12.6%), *Staphylococcus epidermidis* (12%), *E.coli* (12%), and *Enterococcus spp* (11.5%)

Dushyant singh et al. [18] reported, most predominant organisms isolated were *Enterococcus spp* (57.6%) followed by *Staphylococcus aureus* (28.8%), *Streptococcus spp* (21.1%) and *Pseudomonas spp* (15.3%).

Ozer b et al. [19] reported, most predominant organisms isolated were *E.coli* (36.5%) followed by *Pseudomonas spp* (18.9%), *Enterococcus spp* (14.9%), *Staph aureus* (10.8%), *Streptococcus spp* (6.8%), and *Staph epidermidis* (5.4%).

JJ mendes et al. [20] reported, most predominant organisms isolated as *Staph aureus* (51%) followed by *Pseudomonas spp* (12.2 %) and *Acinetobacter spp* (8.2%).

Banashankari et al. [21] reported, most predominant organisms isolated were *Pseudomonas spp* (32%) followed by *Staphylococcus aureus* (19%), *Proteus spp* (18%), *E.coli* (16%), *Staphylococcus epidermidis* (13%) and *Enterococcus spp* (9%)

Antibiotic Sensitivity

Ramani et al. [15]. reported most of the isolates were sensitive to Gentamicin (52.05%), Chloramphenicol (48.43%), Kanamycin (43.49%), Erythromycin (35.8%) and Cephalexin (34.53%) and all the anaerobes were sensitive to Metronidazole.

Grayson ML et al. [22] .compared the efficacy of Imipenem/Cilastatin and Ampicillin/Sulbactum in the treatment of limb threatening foot infection in diabetic patients, found 81% vs. 85% efficacy respectively. Rovan Urbancic V et al. [18]. reported most of the organisms were susceptible to Amoxicillin/Clavulanate (87.9%) and Ciprofloxacin (89.0%).

Chincholikar Dipali A et al. [16] reported that most of the strains were sensitive to cephalosporins and ciprofloxacin. Banashankari et al. [21] reported Enterobacteriaceae group and *P.aeruginosa* strains

were largely susceptible to imipenem (100%), piperacillin-tazobactam, ceftazidime, aminoglycosides, and ciprofloxacin. More than 70% of staphylococcus aureus sensitive to methicillin. Cefoperazone + sulbactum showed about 67% sensitivity, while ciprofloxacin and amikacin were only 23% and 44% sensitive.

In the present study most of the strains were sensitive to imipenem 89% followed by amikacin, ciprofloxacin and Gentamicin.

Conclusion

Diabetic foot ulcers not only cause hospitalization, but also affect the patient economically and may lead to increased mortality. Culture and sensitivity from the wound plays an important role in prescribing the appropriate antibiotic at the time of admission itself rather than starting empirical treatment. The lack of multi-disciplinary approach in the treatment of diabetic foot is quite obvious and there is a lot of scope of improvement in the form of holistic approach to a patient with diabetic foot rather than just treating the foot. Infection control programme and policies should be vigorously pursued in our health care facilities as well as antibiotic prescription regulation to cope with the upsurge of *resistance* to various antibiotics. Thus proper antibiotics policy and measures to restrict the indiscriminate use of antibiotics should be taken to minimize the emergence of drug resistant pathogen, whose spread would leave no option to treat gram negative infections.

Prior publication: Nil

Support: Nil

Conflicts of interest: Nil

Permissions: Nil

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.6

Speciation and Anti-Microbial Susceptibility Pattern of Enterococcal Isolates from Various Clinical Samples with Special Reference to Vancomycin Resistance

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Received on 13.01.2019, **Accepted on** 07.03.2019

Abstract

Background:Enterococci are important causes of both communities acquired and nosocomial infections. They show intrinsic resistance to a number of commonly used antibiotics, particularly the cephalosporins. During the last few years, Enterococci have acquired resistance to a number of important antibiotics including glycopeptides. Enterococci resistant to all three antimicrobial agents (penicillin, aminoglycosides and Vancomycin) pose a serious challenge not only for clinicians but also for health care institutions. It results in treatment failure, selection and spreading of resistant strains in the health care institution. The increasing occurrence of Enterococcusspecies, worldwide, since late 1980s, is of particular concern due to the emergence of Vancomycin Resistant Enterococci (VRE). VRE has also been reported from some parts of India. The appearance of VRE has limited the therapeutic options available for clinicians. *Materials and Methods:* Study was carried out in the Department of Microbiology, Shimogga Institute of Medical Sciences, Shimoga, between November 2015 and October 2018. Enterococci were isolated from various clinical samples at a tertiary care hospital using the standard techniques. The isolated Enterococci are then tested for routine antibiotics sensitivity by disc diffusion method including Vancomycin sensitivity. *Results and Discussion:*A total 330 Enterococcus isolates were obtained from various clinical specimens such as C. Among 330 Enterococcus species, 235 species were Enterococcus faecalis and 95 species are Enterococcus faecium. The Enterococcal species showed 100% sensitivity to Vancomycin and Linezolid. The ability of the laboratory to identify enterococci and to detect Vancomycin resistance promptly and accurately is essential in recognizing VRE colonization and infection and avoiding complex, costly containment efforts that are required when recognition of the problem is delayed. Further, acquisition of Vancomycin resistance leaves few options for therapeutic management.

Keywords: Enterococcus Faecalis; Enterococcus Faecium; Vancomycin; Vre.

How to cite this article:

Siddesh KC, Rakeshgouda Y Patil, Halesh L H. Speciation and Anti-Microbial Susceptibility Pattern of Enterococcal Isolates from Various Clinical Samples with Special Reference to Vancomycin Resistance. J Microbiol Relat Res. 2019;5(1):35-37.

Introduction

Over the last two decades, Enterococci, known only as intestinal commensals with little significance have evolved as deadly pathogens. They are important causes of both communities acquired and nosocomial infections. They show intrinsic resistance to a number of commonly used antibiotics, particularly the cephalosporins. During the last few years, enterococci have acquired resistance to a number of important antibiotics including glycopeptides. Enterococci resistant to all three antimicrobial agents (penicillin, aminoglycosides and vancomycin) pose a serious

challenge not only for clinicians but also for health care institutions. It results in treatment failure, selection and spreading of resistant strains in the health care institution [1].

The present study was aimed to speciate Enterococci which helps to know the prevalent species in and around Shimoga and also, detecting the antimicrobial resistance pattern among Enterococcus isolates obtained from various clinical specimen in a tertiary care hospital I with special emphasis on Vancomycin resistance.

Materials and Methods

The present study was a cross sectional study carried out in the Department of Microbiology, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, during November 2015 and October 2018.Various clinical samples like Urine, Pus, blood and body fluids from the patients attending McGann Teaching Hospital, Shimoga were used for the study. Specimen were collected in a sterile, proper labelled container with aseptic precautions and processed as per standard Microbiological procedures.

All specimens were screened for the pus cells and organism. Specimen was cultured on Blood agar and MacConkey and incubated for 37^0C for 24 hours. Growth was then processed for Gram staining and catalase test. Gram positive cocci arranged in pairs which were catalase negative considered as streptococcus species. Enterococcus isolates were identified and speciation done by their colony morphology, Gram stain and various biochemical tests by standard conventional techniques.

Antibiotic susceptibility testing

The antibiotic resistance profile was determined by Kirby-Bauer disc diffusion method using different antimicrobial agents supplied by manufacturer (HiMedia Laboratories, Mumbai) and interpreted according to guidelines recommended by Clinical and laboratory Standards Institute (CLSI) [5].

Susceptibility to Vancomycin was performed by Kirby-Bauer Disc Diffusion Method (KBDDM) on Mueller Hinton Agar by using 30µg Vancomycin disc (HiMedia) [2].

Results

In the present study, a total of 330 Enterococcus were isolated from various clinical cases. Out of 330 cases, 227 (69%) patients were female and 103 (31%) were male patients. The maximum percentage of isolation was seen among the age group 30-60 years. The sex distribution is shown in Table 1.

Table 1: Gender wise distribution of clinical samples used in the study

Sex	Number	Percent (%)
Male	227	69
Female	103	31
Total	330	100

Out of the total 330 various clinical samples, enterococcus isolated from urine (201), pus / exudates (69), blood (33) and, others (23). Majority of the enterococcus isolates were from urine, followed by pus and then blood (Table 2).

Table 2: Details of various clinical samples from which the enterococcus aureus was isolated.

Clinical Specimen	No. of Enterococcus isolates	Percentage
Urine Pus	201	62.1
Pus	69	20.9
Blood	33	11.8
Others (Body fluids)	23	5.1
Total	330	100

Maximum isolation of Enterococcus isolates was isolated from urine specimen. It indicates that urinary tract infections are the most common infections caused by Enterococci in our hospital.

Among 330 Enterococcus species isolated in our study, 235 species were Enterococcus faecalis and 95 species were Enterococcus faecium (Table 3). Most of the enterococcus isolates were resistant to routinely used antibiotics. All the isolates showed 100% sensitivity to Vancomycin and Linezolid.

Table 3: Species wise distribution of Enterococcus species

Enterococcus spp.	No. of isolates	Percentage
Enterococcus faecalis	235	71.2
Enterococcus faecium	95	28.8
Total	330	100

The percentage of antibiotic sensitivity of Enterococcus species to various antibiotics were differed (Table 4). Enterococcus species showed sensitivity of 35.5% to Ampicillin, 56% to Ciprofloxacin, Norfloxacin (26.4%), Nitrofurantoin (41.3%) and they showed 100% sensitivity for Vancomycin and Linezolid.

Table 4: Percentage of antibiotic sensitivity of Enterococcus species to other antibiotics

Antibiotic	Sensitivity	Resistance
Ampicillin (10µg)	117 (35.5%)	213 (64.5%)
Ciprofloxacin (5µg)	185 (56%)	145 (44 %)

Norfloxacin*	53 (26.4%)	148 (73.6%)
Nitrofurantoin(30µg) *	83 (41.3%)	118 (58.7%)
Vancomycin (30µg)	330 (100%)	00
Linezolid (30µg)	330 (100%)	00

*Antibiotic used for urine samples only (201 samples)

Discussion

Enterococci are important causes of both communities acquired and nosocomial infections. They show intrinsic resistance to a number of commonly used antibiotics. During the last few years, enterococci have acquired resistance to a number of important antibiotics including glycopeptides. The increasing occurrence of enterococcus species, worldwide, since late 1980s, is of particular concern due to the emergence of Vancomycin Resistant Enterococci (VRE). VRE has also been reported from some parts of India. The appearance of VRE has limited the therapeutic options available for clinicians. Imprudent use of antibiotics and colonization pressure are the important causes of the drug resistance in Enterococci. In the present study, 330 Enterococcus isolates from various clinical specimens were used. Out of which maximum number of Enterococci were isolated from urine (62.1%) followed by Pus (21%) and blood. This is slightly lower than Ruoff et al., who isolated maximum number of Enterococci from urine (68.2%). In another study conducted by Talebi et al., maximum number of Enterococci were isolated from urine (85%) followed by Pus (15.5%). Antibiotic resistance among Enterococci is a challenging global problem. Antibiotic resistance seen among Enterococcal isolates may be intrinsic or acquired. In our study, the maximum resistance was observed against Ampicillin (64.3%). In another study carried out by Salem Bekhit et al., (2012) also reported high resistance of Ampicillin accounting for 70.4% resistance among the isolates. Our study has shown ciprofloxacin resistance of 44%, where the study of Sarika Jain et al., (2011) also reported high resistance of ciprofloxacin (75%). In the present study, the highest sensitivity (100%) was shown with Vancomycin and Linezolid among all samples.

Conclusion

In vitro testing of antimicrobial susceptibility of all clinical enterococcal isolates, suitable modification of the usual susceptibility testing procedures, judicial use of antibiotics, systematic surveillance and control of fecalcolonization of resistant enterococci in hospital staff are some of the measures to be adopted for control of the drug resistance in enterococci. The ability of the laboratory to identify Enterococci and to detect Vancomycin resistancepromptly and accurately is essential in recognizing VRE colonization and infection and avoiding complex, costly containment efforts that are required when recognition of the problemis delayed.

Prior publication: No

Support: Nil

Conflicts of interest: None

Permissions: Not applicable

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.7

Isolation, Characterization and Antimicrobial Susceptibility Pattern of *Acinetobacter* Species in Various Clinical Samples

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Received on 18.01.2019, **Accepted on** 07.03.2019

Abstract

Introducion: Acinetobacter is a heterogeneous group of gram negative, oxidase negative, non-motile, non-fermenters emerged as an important nosocomial pathogen causing outbreaks of hospital infections. The high prevalence of multidrug resistant isolates makes initiation of effective empiric treatment challenging. *Objective:* This study was undertaken to isolate and characterise Acinetobacter species in various clinical specimens and to analyse the antibiotic susceptibility pattern. *Materials and Methods:* A total of 5395 clinical specimens were processed in the department of microbiology of a tertiary care hospital over the period of 2 years. Out of which 147 isolates were *Acinetobacter* species. Speciation and antibiotic susceptibility was determined by the standard conventional method. ESBL and MBL production was detected by disc potentiation test method and imipenem EDTA combined disc test, and MBL E-test respectively. *Results:* Prevalence was 2.72%. Most predominant species was *Acinetobacter baumannii* 128 (87.07%). Maximum isolataion was seen among ICU patients (31.97%). Most of strains were resistant to ciprofloxacin (87.5%), ceftazidime (85.94%). All strains were resistant to piperacillin (100%) and sensitive to colistin and polymyxin B. ESBL, MBL production and MDR was detected in 34.01%, 21.77% and 53.06% of the isolates respectively. *Discussion and Conclusion:* A high level of antibiotic resistance was observed in our study and maximum isolation rate of Acinetobacter was in the ICUs associated with respiratory tract infection. *Acineobacter baumani* was the most predominant species. Other species of *Acinetobacter* are also isolated and encountered in hospital acquried infection, though they are sensitive to presently used antimicrobials but in future have potential to acquire resistance. The analysis of susceptibility pattern will be useful in understanding the epidemiology of this organism in our hospital setup, which will help in treating individual cases and controlling the spread of resistant isolates to other individuals.

Keywords: *Acinetobacter* Species; Antimicrobial Susceptibility Pattern.

How to cite this article:
Santosh Mahadevrao Mangalkar, Shital Subhash Rathi, Vijaykumar Virshetty Chincholkar. Isolation, Characterization and Antimicrobial Susceptibility Pattern of *Acinetobacter* Species in Various Clinical Samples. J Microbiol Relat Res. 2019;5(1):39-43.

Introduction

Acinetobacter species are Gram-negative, non-fermentative coccobacilli, these are saprophytic, ubiquitous and have emerged as an important nosocomial pathogen. Among non fermentative organism, it is second most common nosocomial bacteria encountered in clinical specimens [1]. Primary pathogenic role of these bacteria is undoubtedly to cause hospital-acquired infections; mainly among patients at intensive

care units (ICUs). Even cases of community-acquired infections caused by *Acinetobacter* spp. have been reported [2]. *Acinetobacter* causes epidemic outbreaks or endemic occurrence with documented high mortality rates, which is about 25 to 30 % for bacteremia and 40–80% for pneumonia [3,4]. *Acinetobacter spp*. have been implicated in ventilator-associated pneumonia, catheter related blood stream infections, urinary tract infections, cerebrospinal-shunt-related meningitis and wound infections [2].

The most common species to cause infections are *A. baumannii*, followed by *A. calcoaceticus, A. haemolyticus* and *A. lwoffii* [2]. During the last three decades *A. baumannii* isolates have become resistant to more and more classes of antibiotics due to both intrinsic and acquired resistance mechanisms [5,6]. For a long time carbapenems was the most reliable treatment option for infections caused by *Acinetobacter* spp., but carbapenemase-producing isolates are emerging globally [5,7]. Emergence of metallo –β- lactamases (MBL) producing multidrug resistant (MDR) isolates is a matter of concern in an intensive care unit (ICU)[5].

The present study was conducted to find out prevalence of *Acinetobacter* species infection and its antimicrobial susceptibility pattern in various clinical specimens at our hospital, so as to guide the clinicians of our hospital to select appropriate antimicrobial agents and infection control protocol in order to control *Acinetobacter* infection and ultimately for the holistic healthcare.

Materials and Methods

The study was conducted in the Department of Microbiology, Government Medical College and tertiary care hospital, from Dec 2016 to Nov. 2018. A total 5395 specimens like blood, sputum, pus, CSF and other body fluids were subjected to simplified phenotypic identification scheme.

All the samples were subjected to Grams stain except blood and urine and inoculated on blood agar and MacConkey agar medium and incubated at 37^0C for 24 hours. All non-lactose fermenter colonies on MacConkey agar were subjected to gram staining, catalase, oxidase test and motility. *Acinetobacter* are Gram negative bacilli or coccobacilli, oxidase negative, nonmotile and catalase positive. Speciation was done on the basis of citrate utilization test, urea hydrolysis test, arginine hydrolysis, glucose oxidation by oxidation fermentation test, gelatin liquefaction, hemolysis, malonate assimilation and growth at 37^0C and 42^0C. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) as per CLSI guidelines.

ESBL production was detected by using the disc potentiation test method. Ceftazidime, ceftazidime -clavulanic acid and ceftriaxone, ceftriaxone-clavulanic acid discs were used. Production of enzyme Metallobetalactamases (MBL) was detected by using imipenem EDTA combined disc test and MBL E– test.

Results

During the period of study from Dec 2016 to Nov 2018, a total of 5395 specimens were examined from patients of different age group admitted in various medical wards, surgical wards and ICU at Government medical college and tertiary care hospital. A total of 147 isolates were *Acinetobacter* species. Prevalence of *Acinetobacter* species in our study was 2.72%.

Table 1: Ward wise distribution of patients with *Acinetobacter* infection

Ward/ICU	No of patients	Percentage (%)
ICU	47	31.97
Burn ward	36	24.49
Surgery ward	28	19.05
OBGY ward	16	10.89
Medicine ward	11	7.48
Paediatric ward	05	3.40
Ortho ward	04	2.72
Total	147	100

Maximum number of isolates were obtained from patients admitted in ICU (31.97%) followed by burn ward (24.49%). Maximum number of *Acinetobacter* species were obtained from respiratory tract infection 47(31.97%) followed by burn wound (24.49%). In our study, *Acinetobacter baumannii* 128 (87.07%) was predominant species isolated followed by *Acinetobacter lwoffii* 16 (10.89%), *Acinetobacter haemolyticus* 3 (2.04%) (Table 1).

Table 2: Antimicrobial susceptibility pattern of *Acinetobacter baumannii* (N=128)

Drug name	Sensitive	Resistance
Piperacillin	0	128 (100)
Piperacillin-tazobactum	32 (25)	96 (75)
Ampicillin–sulbactum	98 (76.56)	30 (23.44)
Ciprofloxacin	16 (12.5)	112 (87.5)
Levofloxacin	38 (29.69)	90 (70.31)

Tetracycline	20 (15.62)	108 (84.38)
Cotrimoxazole	46 (35.94)	82 (64.06)
Ceftazidime	18 (14.06)	110 (85.94)
Cefotaxime	18 (14.06)	110 (85.94)
Cefepime	27 (21.09)	101 (78.91)
Gentamicin	48 (37.5)	80 (62.5)
Amikacin	78 (60.94)	50 (39.06)
Tobramycin	78 (60.94)	50 (39.06)
Imipenem	87 (67.97)	41 (32.03)
Meropenem	87 (67.97)	41 (32.03)
Colistin	128 (100)	0
Polymyxin	128 (100)	0

Figures in parenthesis shows percentage

All isolates of *Acinetobacter baumannii* were resistant to piperacillin. Most of the isolates were resistant to ciprofloxacin (87.5%), ceftazidime (85.94%) and cefotaxime (85.94%), tetracycline (84.38%), cefepime (78.91%). All strains were sensitive to colistin and polymyxin B (Table 2).

Table 3: Distribution of *Acinetobacter* isolates according to ESBL, MBL production and multidrug resistance

Acinetobacter spp.	ESBL positive	MBL positive	MDR strains
Present	50 (34.01)	32 (21.77)	78 (53.06)
Absent	97 (65.99)	115 (78.23)	69 (46.94)
Total	147	147	147

In our study, 34.01% of *Acinetobacter* isolates were ESBL producer and 21.77% MBL producer and 53.06% were multidrug resistant isolates (Table 3).

Discussion

Acinetobacter species are emerging as an important organism causing hospital acquired infections [8]. These organisms cause serious health care associated infections as well community acquired infections [9,5].

Acinetobacter baumannii is the most common clinically important bacteria belonging to this genus [9,5] which causes epidemic outbreaks or endemic occurrence with documented high mortality rates [2,11] and outbreaks have also been reported from India [4]. The mortality rate of nosocomial infections caused by *A.baumannii* is relatively high, i.e. 25 to 30% for bacteremia and 40–80% for pneumonia [3]. In our study, a total 147 isolates of *Acinetobacter* species were isolated. Prevalence of *Acinetobacter* species in our study was 2.72% which is lower as compared to various studies (4 to 9%) [12,13,14]

In the present study, maximum number of isolate were from ICU (31.97%) followed by Burn ward (24.49%) and Surgery ward (19.05%) (Table no.1) which is similar to the study of Gupta N et al. [15] (2015). ICU infections more because of opportunities for cross transmission, immune-compromised patients who are colonized and having indwelling devices, heavy use of broad spectrum antibiotics and frequent contamination of the hands of health care workers during patient care. The development of ICU-acquired infections is strongly related to prolonged ICU stay.

Isolation of *Acinetobacter* species was maximally from respiratory tract infection (31.97%) followed by burn wound infection (24.49%) which is similar to study done by Singla et al. [16]. (2013) Amandeep Kaur et al. [17] (2016), Jaggi et al. [18] (2012).

In our study, *Acinetobacter baumannii* (87.07%) was predominant species isolated which is similar to studies done by Dash et al. [19] (2013) (79.6%). Somewhat lower isolation rate was seen in study done by Tripathi et al. [20] (2014) (74.50%), Mostofi et al. [14] (2011)(71%) and Singla et al. [16] (2013) (74.6%) as compared to our study.

One of the most striking feature of genus *Acinetobacter* is the ability to develop antibiotic resistance extremely rapid in response to challenge with new antibiotics. In our study, all isolates *of A.baumannii* were resistant to piperacillin, 87.5% resistant to ciprofloxacin. Resistance to cefotaxime and ceftazidime was 85.94% and carbapenam resistance was 32.03%. All isolates were sensitive to colistin and polymixin B (Table 2). As compared to our study, Gupta N et al. [15] (2015) found lower resistance pattern to piperacillin (55%). resistant to ciprofloxacin (23%) lower resistance to ceftazidime (46%) and cefotaxime (43%). carbapenam resistance (22%).

As per table 3, ESBL production was seen in 34.01% isolates which is comparable to study done by Gupta N et al. [15] (2015) (31.5%) while Kansal et al. [21] (2009) found maximal ESBL producing isolates in their study (75%). MBL production was 21.77% which is similar to Kumar et al. [22] (2011) (21%) while somewhat lower incidence was seen in a study done by Gupta N et al. [15] (2015) (14.4%). Multidrug resistance in our study was 53.06% which is comparable to study done by Mostofi et al. [14] (2011) and Dash M et al. [19] (2013) who found 54% and 54.7% of strains as multidrug resistant

respectively

The resistance patterns detected in *Acinetobacter* could reflect the antibiotic misuse and lack of regulations on drug use. Resistance to various antimicrobials agent limits the selection of appropriate drugs for the effective management making difficult to control and treat. However, as the resistance against colistin and polymyxin is not very high in our country, it can still be used as the drug of choice against multidrug resistant strains of *A.baumannii*.

Conclusion

The high prevalence of multidrug resistant *Acinetobacter* species in our hospital only underscores the urgent need for instituting control measures to limit the spread of this troublesome nosocomial pathogen in hospital areas. Definitive identification and characterization of *Acinetobacter* species by simple phenotypic methods can be used. Rationale use of antibiotics is important and necessary to prevent microbial resistance catastrophe. A continued awareness of the need to maintain good housekeeping and control of the environment, including equipment decontamination, strict attention to hand washing should undertake to control the spread of *Acinetobacter* in hospitals.

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Original Article

Journal of Microbiology and Related Research
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.5119.8

Vancomycin Resistance among Methicillin Resistant *Staphylococci* Isolated from Different Clinical Samples at Tertiary Care Hospital

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Received on 29.01.2019, **Accepted on** 07.03.2019

Abstract

Background: The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains exhibiting two different resistance mechanisms. The emergence and spread of resistance to vancomycin is a threat to the already challenging therapy of MRSA. *Materials and Methods:* The present study was carried out to find out the presence of VISA and VRSA in the tertiary hospital. A total 570 *staphylococcus aureus* isolates consisting of 340 MRSA and 230 MSSA were isolated from different clinical specimens from various outpatient departments and wards using the standard techniques. All MRSA isolates were subjected to disc diffusion testing and MIC testing against vancomycin. *Result and Discussion:* Out of the 340 MRSA isolates, 270 isolates were Vancomycin sensitive *Staphylococcus aureus* (VSSA) (MIC: 0.5-2µg/ml), 70 isolates were Vancomycin intermediate *Staphylococcus aureu*s (VISA) (MIC: 4-8µg/ml) and none were Vancomycin resistant *Staphylococcus aureus* (VRSA) (MIC: >16µg/l). The present study reveals for the first-time emergence of VISA/VRSA from this part of Karnataka, India.

Keywords: *Staphylococcus Aureus*; MRSA; Vancomycin; VRSA; VISA.

How to cite this article:
Siddesh KC, Ashrith KC, Halesh L H. Vancomycin Resistance among Methicillin Resistant *Staphylococci* Isolated from Different Clinical Samples at Tertiary Care Hospital. J Microbiol Relat Res. 2019;5(1):45-49.

Introduction

The emergence of high levels of penicillin resistance followed by the development and spread of strain resistant to the semisynthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides has made the therapy of *staphylococcal* disease a global challenge [1]. *Staphylococcus aureus*, a major cause of potentially life-threatening infections acquired in health care and community settings, has developed resistance to most classes of antimicrobial agents. A dramatic increase in the number of health care-associated infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) in the 1990s and the recent emergence of MRSA in community-associated infections highlight the success of this species as a pathogen and its ability to adapt under pressure from antimicrobial agents [2].

Methicillin resistant *S.aureus* (MRSA) was first detected in 1961 and has occurred in many countries. Even after 40 years it is still among the top three

clinically important pathogens [3,4]. However, in recent years, clinicians have been concerned by the increased frequency of MRSA infections.

The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA [5,6]. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant Staphylococcus aureus (VRSA) strains exhibiting two different resistance mechanisms.

Initially vancomycin intermediate *Staphylococcus aureus* (VISA) noted in Japan in 1996 and subsequently in United States in 1997 was believed to be due to the thickened cell wall [7,8]. The second, noted in United States in 2002 [9] among *Staphylococcus aureus*, was identical to the mechanism seen in vancomycin-resistant enterococcus [10]. Vancomycin resistant Enterococcus faecium harbors the vanA operon, which contains five genes, VanS, -R, -H, -A and –X [10]. But Tiwari and Sen have reported a VRSA which is van gene-negative11. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil [12], France [13], United Kingdom [14], Germany [15], India [11,16] and Belgium [17] has confirmed that the emergence of these strains is a global issue.

Materials and Methods

The present study was a cross sectional study carried out in the Department of Microbiology, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, during August 2014 to July 2017. Various clinical samples like pus, blood and urine from the patients attending outpatient Departments and from those admitted to wards of Surgery, Medicine, Orthopedics and Obstetrics and Gynecology in McGann Teaching Hospital, Shimoga were used for the study. Specimen were collected in a sterile, proper labelled container with aseptic precautions and processed as per standard Microbiological procedures.

Staphylococcus aureus isolates were identified by their colony morphology, Gram stain and various biochemical tests by standard conventional techniques. Methicillin resistant *Staphylococcus aureus* (MRSA) strains were identified by phenotypic methods by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Disc diffusion method

Vancomycin resistance was detected by disc diffusion method using Vancomycin discs (30 µg) as per CLSI guidelines and interpreted as per the manufacturer guidelines (Hi-Media Laboratories, Mumbai)

Determination of MIC by agar diffusion methodusingHicomb strips (Hi-Media Laboratories private limited, Mumbai):

- This strip is intended only for agar diffusion method and not for broth dilution method
- Plates were prepared with Mueller-Hinton agar for rapidly growing aerobic organism as per Kirby-Bauer method. Only pure cultures were inoculated.
- A sterile non-toxic cotton swab on a wooden applicator was dipped into the standardized inoculum and the soaked swab was firmly rotated against the upper inside wall of the tube to express excess fluid.
- The entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between each streaking.
- The inoculum was allowed to dry for 5-15 minutes with lid in place.
- The HiComb MIC strip was applied to the agar surface with MIC scale facing upwards as per manufacturer's instructions. Once applied the strip was not moved and was let to absorb to the surface of agar media.
- The agar plate was incubated at 35-37°C and examined after 18-24 hours.

Interpretation

The zone of inhibition was in the form of an ELLIPSE. MIC value would be the value at which the zone convenes the comb like projections of the strips and not at the handle.

- If there were no zone of inhibition observed, the MIC was reported as greater than the highest concentration on the strip.
- If zone of inhibition was below the lowest concentration then the MIC was reported as less than the lowest concentration.Readings were taken according to manufacturer's instructions as MIC of ≤ 2µg/ml - Susceptible (VSSA), MIC of 4-8µg/ml - Intermediate (VISA) and MIC of ≥ 16µg/ml - Resistant (VRSA).

Results

In the present study, a total of 550 clinical samples were collected from various clinical cases. Out of 550 cases, 297 (54%) patients were male and 253(46%) were female patients. The sex distribution is shown in Table 1.

Table 1: Gender wise distribution of clinical samples used in the study

Gender	Number	Percent (%)
Male	297	54
Female	253	46
Total	550	100

Out of the total 550 various clinical samples, namely from pus/exudates (396), blood (33) and urine (121), 313 were *Staphylococcus aureus*. Majority of the *S. aureus* isolates were from pus followed by blood and then urine.

Of the 313 *Staphylococcus aureus* isolates, 187 (59.75%) were Methicillin Resistant *Staphylococcus aureus* and 126 (40.25 %) were Methicillin Sensitive *Staphylococcus aureus*. Of the 187 MRSA isolates, 183 were found in pus and 4 were found in blood whereas none were found in urine.

Table 2: Details of various clinical samples from which the *staphylococcus aureus* was isolated.

Clinical Specimen	No. of samples taken	*Staphylococcus aureus*	
		Number	Percentage
Pus	396	298	95
Blood	33	09	3
Urine	121	06	2
Total	550	313	100

In the present study, out of 187 Methicillin Resistant *Staphylococcus aureus* (MRSA), all the isolates were susceptible vancomycin by disc diffusion method with varying zone of inhibition. Minimum inhibitory concentration was calculated by HiComb MIC test method (HiMedia Laboratories Pvt. Limited). Out of the 187 MRSA isolates, 148 isolates were Vancomycin sensitive *Staphylococcus aureus* (VSSA), 39 isolates were Vancomycin intermediate *Staphylococcus aureus* (VISA) and none were Vancomycin resistant *Staphylococcus aureus* (VRSA). All 39 VISA isolates were isolated from pus and exudates.

Antibiotic susceptibility pattern showed that all MRSA strains were resistant to oxacillin and cefoxitin. Also, they were resistant to most of the antibiotics tested and all MRSA strains were susceptible to vancomycin

Table 3: Specimen wise distribution of MRSA

Clinical specimen	MRSA	
	Number	Percentage
Pus / Exudates	183	97.86
Blood	04	2.14
Urine	0	0
Total	187	100

Discussion

In the present study 550 clinical samples were collected. Out of which 396 samples were pus, 121 urine samples and 33 were blood samples.Of the 313 *Staphylococcus aureus* isolated, 187 (59.75%) were found to be methicillin resistant.

The reported percentage of MRSA isolation from clinical specimens by different workers varies over a wide range as shown in the table 4.

In a study conducted by Thati et al in Hyderabad, out of 358 clinical isolates of *Staphylococcus aureus*, 285 (79.6%) were identified as Methicillin resistant *Staphylococcus aureus* (MRSA) by disc diffusion method. Our study has also shown MRSA incidence of more than 50%.In another study conducted by Indian Network for Surveillance of Antimicrobial Resistance (INSAR), the percentage of MRSA isolated among *Staphylococcus aureus* isolates was 41%.Tiwari et al. (2006), Institute of Medical Sciences, Banaras Hindu University, Varanasi found out the presence of VISA and VRSA in the northern part of India, the percentage of MRSA isolated for a period of three years from August 2002 to July 2005 was 40.61%. Present study has shownhighest numbers of MRSA (183) are isolated from pus followed by blood. Chakravarthy A et al [19], Mehta A.P.et al [20] and Pal N. and Ayyagiri A. [21] have also reported maximum number of MRSA isolation from pus. Allthe MRSA isolates were resistant to penicillin and Ampicillin. MRSA isolates were significantly more resistant (in numbers) to all the tested antibiotics except vancomycin and linezolid. The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *S. aureus* (VRSA) strains.

In the present study, vancomycin susceptibility was detected by Kirby-Bauer's disc diffusion method and MIC was determined by HiComb MIC test (Himedia Laboratories, Mumbai, India). All strains were susceptible to vancomycin by disc diffusion method.

Table 4: Percentage of MRSA samples used by various authors in the previous reports.

Sl. No.	Author	Place	Year	% MRSA
1.	Furuno et al. [22]	Baltimore, USA	2003	9.83
2.	Tiwari et al. [11]	Varanasi, India	2006	40.61
3.	Indian Network for Surveillance of Antimicrobial Resistance (INSAR) [21]	India	2009	41.00
4.	Thati et al. [10]	Hyderabad, India	2011	79.60
5.	Present Study	Shimoga, India	2017	59.65

Table5: Percentage of VSSA samples used by various authors in the previous reports.

Sl.No.	Author	Place	Year	VSSA (%)
1.	Assadullah et al. [16]	Srinagar, India	2003	81.70
2.	Reuf C. [26]	USA	2004	77.00
3.	Tiwar et al. [11]	Varanasi, India	2006	97.48
4.	Thati et al. [10]	Hyderabad, India	2009	93.57
5.	Present Study	Shimoga, India	2017	79.14

Table 6: Percentage of VISA samples used by various authors in the previous reports.

Sl.No.	Author	Place	Year	%VISA
1.	Assadullah et al. [16]	Srinagar, India	2003	15.00
2.	Reuf C. [26]	USA	2004	20.00
3.	Tiwar et al. [11]	Varanasi, India	2006	1.89
4.	Thati et al. [10]	Hyderabad, India	2009	4.47
5.	Present Study	Shimoga, India	2017	20.85

Widespread use of vancomycin to treat infections caused by MRSA has been reported to result in the emergence of low-level resistance. Vancomycin was used clinically in treating infections in our hospital during study period. This could be the reason for detecting vancomycin resistance in MRSA isolates in the present study.

Previous studies done by Assadullah et al, Tiwari et al, Thati et al have reported 81.7%, 97.48% and 93.5% susceptibility to Vancomycin. 79.41% susceptibility to Vancomycin was found in the present study (Table 5). In the present study, 39isolates (20.85%) showed MIC of 4-8 µg/ml which were identified as Vancomycin Intermediate *Staphylococcus aureus* (VISA, Table 6).

Conclusion

Despite the recent reports that gram negative bacterium has overtaken *staphylococci* because the leading explanation for health facility infections, MRSA continues to be the main threat in the health care setting.

Prior publication: No

Support: Nil

Conflicts of interest: None

Permissions: Not applicable

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