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Original Article

Microbiology Section

Aerobic Bacterial Pathogens and their Antimicrobial Susceptibility Pattern in a Tertiary Care Centre from Kanchipuram District, Tamil Nadu, India- A Retrospective Study

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ABSTRACT

Introduction: The infections of wound play an important role in delaying the healing of wound. Moreover, the widespread uses of antibiotics, together with the length of time lead to the emergence of resistant bacterial pathogens contributing to morbidity and mortality. So, there is a need for understanding the distribution of pathogens and the susceptibility pattern of the locality which becomes crucial in the treatment of wound infections.

Aim: To determine the frequency and distribution of bacterial isolates and their drug susceptibility pattern isolated from inpatients and outpatients with pus and wound discharge.

Materials and Methods: This retrospective study was conducted in the Department of Microbiology at Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research, Kanchipuram, Tamil Nadu, India, from May 2018 to April 2019. The pus samples were collected from the patients who visited outpatient department and were admitted at In Patient Department (IPD) in the hospital with skin and soft tissue infection. Bacteria were identified by culture and biochemical tests and antibiotic susceptibility test was done by disc diffusion method. Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta Lactamase (ESBL) in gram negative isolates were detected by cefoxitin disc diffusion method and double disc diffusion method respectively.

Results: *S.aureus* (28.4%) was the most prevalent pathogen followed by *P.aeruginosa* (20.6%), *Proteus* (15%), *Klebsiella* (11.6%), *Acinetobacter* (8.3%), *E.coli* (7.6%), *Enterococcus* sp. (4.8%), *S.pyogenes* (3.3%). *S.aureus* and *S.pyogenes* were sensitive to vancomycin and linezolid. MRSA was found in 61% of isolates and of 61%, 13% of isolates showed inducible clindamycin resistance. *Klebsiella*, *Pseudomonas* and *Acinetobacter* isolates were resistant to multiple antibiotics including third generation cephalosporins. ESBL production was observed in 47% and 31% of *Klebsiella* and *Pseudomonas* isolates, respectively. The highest carbapenemase production was found in 21% of *Acinetobacter* sp.

Conclusion: The findings of this study clearly help us to understand the developing resistance percentage among the bacteria causing wound and soft tissue infections and a need for the judicial use of antibiotics, an updated antibiotic policy for the hospital and practice of strict hospital infection control measures.

Keywords: Antimicrobial resistance, Bacterial isolates, Exudates, Pus

INTRODUCTION

The skin and soft tissue infections are those bacterial infections which occur as a result of loss in skin integrity. This can happen because of road traffic accident, iatrogenic procedures like injections or surgical incisions, etc. Such infections can result in exudative fluid production which is usually made of dead White Blood Cells (WBCs) and tissue debris [1]. Such infections is caused by aerobic and anaerobic bacteria which have influenced on the prognosis of the patient in terms of hospital stay because of increased morbidity, loss of parts leading to disability, inability to go back to work and the financial issues [2]. The common bacterial pathogens responsible for wound infections are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and bacteria belonging to family Enterobacteriaceae [3]. Treatment of wound infections has posed a great challenge to the treating physicians and surgeons. This can be attributed mainly, because of emerging resistance to antibiotics among previously susceptible bacteria and to the Multidrug Resistant (MDR) bacteria like MRSA and ESBL [4]. Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. Though as a part of evolution of bacteria, a small percentage of resistance will always occur, still the issue of concern is about the major percentage of resistance which is because of the empirical use of antibiotics that is started without culture and sensitivity report and the counter sales of antibiotics [5]. Monitoring of resistance patterns in the hospital is needed to overcome these difficulties and to improve the outcome of serious infections in hospital settings. During the last few decades, MDR gram negative organisms and MRSA were increasingly associated with pus infections under hospital settings due to extensive overuse and inadequate dose regimen of antibiotics [6]. Since these MDR bacteria are resistant to usual antibiotics, there occurs a limitation in the antibiotic treatment of such bacteria as there are only a few options available. This becomes a major hindrance in the health outcome all over the world which should definitely be sorted out [7]. One proper solution for this issue would be to completely do surveillance and understand the distribution of the bacteria in various exudative samples and the pattern of their susceptibility to the first line and second line antibiotics. This particular study will be more useful in a way that it has been conducted in a tertiary care institute in a rural setting which may influence certain changes in the pattern of isolates also.

So, the aim is to study was to determine the frequency of bacteria and their susceptibility profile in all the exudative samples from the outpatients as well as inpatients.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Microbiology at Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research, Kanchipuram, Tamil Nadu, India, from

1st May 2018 to 30th April 2019. Institutional Ethics Committee approval was obtained (MAPIMS/IEC/52/2019). All the patients who presented with skin and soft tissue infection during the study period were selected as study population. The pus samples were collected from the patients who visited outpatient department and also from patients who were admitted at inpatient department. Samples were collected from skin (furuncles, pustules, and abrasions), nasal wounds, ears, legs and were processed for gram staining and culturing. The samples were aseptically inoculated on blood agar (with 5% sheep blood) and MacConkey's agar plates, incubated aerobically at 35°C-37°C for 24-48 hours [8]. Identification and characterisation of isolates were performed on the basis of gram staining, microscopic characteristics, colony characteristic and biochemical tests using standard microbiological methods [8].

Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method [9]. Antibiotics discs containing penicillin (10 U), amoxicillin (30 µg), cefoxitin (30 µg), cefazolin (30 µg) amikacin (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), ciprofloxacin (10 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), linezolid (30 µg), meropenem (10 µg), piperacillin/tazobactum (100/10 µg), ceftazidime/clavulanic acid (100/10 µg), tetracycline (30 µg) and vancomycin (30 µg) were obtained from Himedia Laboratories (Mumbai, India) and used as per manufacturer's instructions. Antibiotic susceptibilities of bacterial isolates were determined according to the method recommended by the Clinical and Laboratory Standards Institute guidelines [9]. MRSA detection was done using cefoxitin disk and isolates showing zone diameter of ≤21 mm were considered as MRSA, ESBL detection was done using double disk diffusion method with combination disk, Carbapenam Resistant Enterobacteriaceae (CRE) using imipenem and Carbapenam disc diffusion and Inducible Clindamycin Resistance was detected using D test in all isolates of *Staphylococcus aureus* according to CLSI 2018 guidelines [9].

D test [9]

Erythromycin disc (E) was placed at a distance of 15 mm from Clindamycin Disc (CD) on a Mueller Hinton agar plate previously inoculated with bacterial suspension and overnight incubation was done at 37°C. The following phenotypes were categorised:

1. Inducible MLSB (iMacrolide, Lincosamide and Streptogramin B) phenotype- The isolates showing resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D-shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.
2. Constitutive MLSB (cMLSB) phenotype- The isolates which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape of zone of inhibition if any around clindamycin.
3. MS phenotype (MS)- The isolates exhibiting resistance to erythromycin (zone size ≤13 mm) while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin.

STATISTICAL ANALYSIS

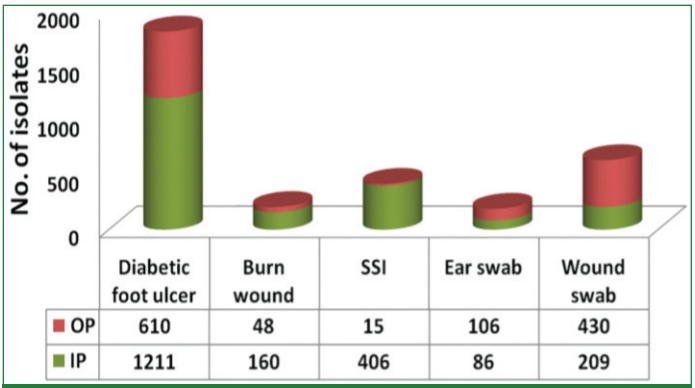
The data were collected and entered in Microsoft Excel sheet. They were analysed using Epi Info software. The frequency, mean and percentage were calculated to know the distribution pattern and the prevalence rate.

RESULTS

A total of 3281 samples received in the microbiology laboratory were analysed. The age of the patients varied between 2 years to 75 years and the mean age was 45 years [Table/Fig-1]. A total of 1982 of them were males and 1299 were females. The male: female ratio was 1.52:1. The highest contribution of pus was from the diabetic foot ulcer (55.5%) followed by wound swab (19.4%) [Table/Fig-2]. Outpatient department contributed to 36.8% (1209) samples. Of 3281 samples, 2529 isolates of gram positive and gram negative bacteria were grown after aerobic culture. Gram-negative bacteria were the dominant isolates N=1601 (63.31%) from samples that showed growth (N=2529) compared to gram-positive bacteria 928 (36.69%)). *S.aureus* (28.4%) was the most frequent isolate followed by *Pseudomonas* spp. (20.6%), *Proteus* spp. (15%), *Klebsiella* spp. (11.6%), *Acinetobacter* spp. (8.3%), *E.coli* (7.6%), *Enterococcus* spp. (4.8%) and *Streptococcus pyogenes* (3.3%). The distribution of isolates among inpatient and outpatient is given in [Table/Fig-3].

Age group (years)	Number	Percentage
≤10	92	2.8
11-20	327	9.9
21-30	575	17.5
31-40	142	4.3
41-50	1021	31.1
51-60	720	21.9
≥60	404	12.3

[Table/Fig-1]: Age distribution (N=3281).



[Table/Fig-2]: Distribution of samples (N=3281).

Organisms	Diabetic foot ulcer		Burn wound		SSI		Ear swab		Wound swab		Total no. of isolates
	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	
S.aureus MSSA	84	67	23	1	52	1	7	12	11	23	281 (11.1%)
S.aureus MRSA	210	82	22	6	45	1	3	9	17	44	439 (17.3%)
GAS	72	0	0	0	13	0	0	0	0	0	85 (3.3%)
Enterococcus	53	14	7	1	11	0	0	0	0	37	123 (4.8%)
E.coli	45	45	11	5	7	0	0	0	27	53	193 (7.6%)
Klebsiella	93	62	19	4	14	0	0	0	25	78	295 (11.6%)
Proteus	189	59	17	3	33	0	9	15	12	43	380 (15%)
Acinetobacter	78	35	6	5	19	0	0	12	15	42	212 (8.3%)
Pseudomonas	201	96	24	15	48	8	14	30	27	58	521 (20.6%)

[Table/Fig-3]: Distribution of bacterial isolates from pus samples showing growth (N=2529).
IP: Inpatient; OP: Outpatient; GAS: Group A *Streptoccoccus*; MRSA: Methicillin resistant *Staphylococcus* aureus; MSSA: Methicillin susceptible *Staphylococcus* aureus

The antimicrobial susceptibility patterns of the gram-positive and gram-negative bacterial isolates is presented in [Table/Fig-4]. The predominant isolate was *S. aureus* which revealed high level of resistance to penicillin (92%) followed by erythromycin (49%). MSSA and MRSA were observed in 39% and 61% of isolates, respectively. The percentage of CRE was 14.9% [Table/Fig-5]. Inducible clindamycin resistance was observed in 57 isolates (13%) and 22 isolates (8%) of MRSA and MSSA isolates respectively. cMLSB phenotype R to E and CD was observed in 20% and 11% isolates of MRSA and MSSA isolates respectively [Table/Fig-6]. Among the isolates of *S.pyogenes*,

Antibiotics	*S. aureus*	GAS	*Enterococcus*	*E.coli*	*Klebsiella*	*Proteus*	*Acinetobacter*	*Pseudomonas*
Penicillin	662 (92)	7 (8)	15 (12)	-	-	-	-	-
Amoxicillin	-	-	14 (11)	-	-	-	-	-
Piperacillin/Tazobactum	-	-	-	21 (11)	68 (23)	46 (12)	57 (27)	203 (39)
Cefoxitin	438 (61)	-	-	-	-	-	-	-
Cefazolin	230 (32)	-	-	-	-	-	-	-
Cefuroxime	-	-	-	-	-	-	-	-
Ceftazidime	-	-	-	89 (46)	165 (56)	118 (31)	81 (38)	208 (40)
Ceftriaxone	-	0	-	44 (23)	139 (47)	46 (12)	61 (29)	162 (31)
Gentamycin	324 (45)	-	0 (HLG)	21 (11)	53 (18)	57 (15)	42 (20)	146 (28)
Amikacin	0	-	10 (8)	44 (23)	27 (9)	42 (11)	40 (19)	99 (19)
Ciprofloxacin	173 (24)	-	-	42 (22)	38 (13)	91 (24)	72 (34)	188 (36))
Clindamycin	274 (38)	8 (10)	15 (12)	-	-	-	-	-
Chloramphenicol	51 (7)	9 (11)	-	-	-	-	-	-
Erythromycin	353 (49)	14 (16)	7(6)	-	-	-	-	-
Linezolid	0	0	0	-	-	-	-	-
Vancomycin	0	0	0	-	-	-	-	-
Imipenem	-	-	-	25 (13)	68 (23)	53 (14)	32 (15)	52 (10)
Meropenem	-	-	-	21 (11)	44 (15)	65 (17)	45 (21)	73 (14)

[Table/Fig-4]: Resistance profile of the bacterial isolates from pus N (%).

Organisms	MRSA (%)	ESBL (%)	Carbapenem resistant (%)
S.aureus	439 (61)	-	-
E.coli	-	44 (23)	21 (11)
Klebsiella	-	139 (47)	44 (15)
Proteus	-	46 (12)	65 (17)
Acinetobacter	-	61 (29)	45 (21)
Pseudomonas	-	162 (31)	73 (14)

[Table/Fig-5]: Distribution of MRSA, ESBL and Carbapenem resistant gram negative bacteria N (%).

the highest resistance percentage was towards erythromycin followed by chloramphenicol. All Gram positive isolates were sensitive to vancomycin and linezolid.

	iMLSB phenotype R to E and S to CD (D-test positive)$_1$	cMLSB phenotype R to E and CD (D test negative)$_2$	MS phenotype$_3$
MRSA (61%)	57 (13)	88 (20)	40 (9)
MSSA (39%)	22 (8)	31 (11)	6 (2)

[Table/Fig-6]: D test interpretation among *S.aureus* N (%).

Majority of Gram negative isolates were susceptible to imipenem, meropenem. Gram negative isolates were most resistant to third generation cephalosporins. Among Enterobacteriaceae, the highest resistance was observed towards cephalosporins, ciprofloxacin and aminoglycosides. Carbapenem resistance was observed in 15.5% of all GNB isolates. Of non-fermentors, the second predominant gram-negative isolate, *P.aeruginosa* showed high level resistance to ceftazidime (40%), piperacillin/tazobactum (39%), ciprofloxacin (36%) and ceftraixone (28%).

DISCUSSION

Pyogenic infections are characterised by inflammation with pus formation and it can be endogenous or exogenous. When a wound becomes infected and fails to heal, the patient suffers from morbidity and treatment costs. In this study, majority of pus sample belonged to diabetic foot ulcer and this may be due to the compromised immune system in diabetes mellitus which leads to secondary infection. Persons with diabetes are often exposed to several antibiotics which increase their risk of developing MDR infection [10]. The findings of this study indicate the predominance of *S.aureus* followed by *Pseudomonas* and this is in agreement to the study reported previously [11-13]. Both *S.aureus* and *Pseudomonas* often forms the biofilm in the chronic ulcers which makes these organisms develop resistant towards the antibiotics. *S.aureus* is usually detected in the top layer of wounds, while *P. aeruginosa* is localised in the deepest region of wound bed [14]. Antibiotic susceptibility results revealed that a high degree of resistance was seen for majority of the bacterial isolates. For gram positive bacteria, vancomycin, linezolid and amikacin were found to be the most effective antibiotics. In this study, MRSA was found in 61% of isolates which is very high when compared to a study done in 2016 in same locality [15] which showed only 19% and the prevalence of MRSA is varying over the time: for instance, 52.9% in 2001 [16], 31.8% in 2006 [17], 42% and 40% in 2008 and 2009, respectively [18]. Inducible clindamycin resistance was observed in 13% and 8% of MRSA and MSSA isolates and the similar result were reported earlier [19,20].

The degree of resistance was even higher among the gram negative bacteria and the commonly used drugs were found to be more resistant with an average resistance ranging from 50% to 100%. Meropenem, piperacillin-tazobactam and amikacin were found to be the most effective antimicrobial agents which was in accordance with a study in North India [1] and with the finding of Subha et al., [21]. *Pseudomonas* was isolated higher in the present study which differs from the study of Sudhaharan S et al., done at Telengana which shows *E.coli* as the highest percentage isolate [22]. But it was similar to other study findings done in a nearby locality of our hospital [23]. ESBL production was higher in the *Klebsiella* sp. This was in accordance to the findings of a study done in the same district [21]. Carbapenemase production was higher in *Acinetobacter* and *Proteus*. This was in contradiction to the findings of two other studies done at Bahrain and China where Carbapenemase production was higher in *Klebsiella pneumonia* [24,25]. The change in the prevalence percentage and antibiotic sensitivity patterns among various countries as discussed can be due to the geographical and environmental conditions. This study being conducted in a rural area shows difference in these patterns from studies conducted in other tertiary care institutes itself which can be attributed to the over counter antibiotic prescription and the practice of incomplete antibiotic course by the people [24,25]. Increase in the percentage of MRSA from a study of 2016 conducted in same district is an alarming sign [15]. The distribution and prevalence of MRSA and ESBL has been compared with other studies of same locality and tabulated. ESBL producing *Klebsiella* spp has shown a marked rise which is a noteworthy sign [Table/Fig-7]. Existence of high drug resistance to multiple antibiotics in *E. coli*, *S. aureus*, *K. pneumoniae*

and *P. aeruginosa* isolates from pus samples in this study and several other related reports [3,26] makes us aware about the irrationality in the use of antibiotics and a major breach in the hospital infection control policies. More education has to be provided to the clinicians regarding the judicial use of antibiotics with an updated antibiotic policy [22].

Study	Year of publication	MRSA	ESBL producing Enterobacteriaceae	Distribution of ESBL
Jayachandran AL et al., [15]	2016	19%	Not studied	Not studied
Subha M and Srinivasagam M [21]	2018	17.5%	42.10%	*E.coli*-23.6% *Klebsiella* .25%
This study	2021	61%	26.38%*	*E.coli*-23% *Klebsiella*-47%

[Table/Fig-7]: Comparison of MRSA and ESBL prevalence and distribution with studies from other tertiary care institutes of same locality [15,21].
The ESBL producing Enterobacteriaceae in this study namely were *E.coli*, *Klebsiella* spp and *Proteus* spp*

Limitation(s)

The study would have been more useful in finding the epidemiology of infections if molecular detection was performed to find out resistance genes. Also, the combined phenotypic methods like screen agar, Minimum Inhibitory Concentration (MIC) determination, Modified hodge test along with disc diffusion if done would have added more evidence for detection of MRSA, ESBL and CRE. This study was taken up for the purpose of preparing an antibiotic policy for the hospital and hence, the objectives were limited to only determination of frequency and percentage of susceptibility by disc diffusion. This will be expanded with more objectives including molecular studies in the future.

CONCLUSION(S)

The study adds more evidence of developing resistance among the bacteria causing skin and soft tissue infections. Infact the changing resistance patterns in the same locality have been discussed which poses a more serious concern. This calls for a more serious hospital infection control policy including an updated antibiotic policy for the hospital and restricted and judicial use of antibiotics by the practitioners. In the future, this study will be used to frame an updated antibiotic policy with the support of data from samples other than pus and molecular study included with it.

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