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Bacterial and Antimicrobial Susceptibility Profile of Skin and Soft Tissue Infections among Patients Attending the Tertiary Health Care Set Up

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ABSTRACT

Introduction: Skin and soft tissue infections (SSTIs) have variable etiology and clinical presentation. Emergence and spread of antibiotic resistance in organisms causing skin and soft tissue infections is posing a great therapeutic challenge. It is important to monitor the changing trends in bacterial infection and their antimicrobial susceptibility pattern to provide appropriate antimicrobial therapy for controlling infection, preventing morbidity and improve the quality of life.

Aims: To determine the bacterial etiology and their antimicrobial susceptibility pattern of soft tissue infections among patients attending the tertiary care set up.

Materials and Methods: Pus samples received in the Department of Microbiology were included in the study. Isolation and identification were done as per standard laboratory protocol. Antimicrobial susceptibility test was done as per CLSI guidelines to determine the various resistance mechanism such as Methicillin-resistance in S. aureus, HLAR in Enterococcus spp. and ESBL production in Escherichia coli and Klebsiella spp.

Results: Out of 1672 pus samples received, 1088 (65.1%) bacterial isolates were obtained. 674 (61.9%) were Gram-negative bacteria, 398 (36.6%) were Gram-positive bacteria and 16 (1.6%) were gram positive bacilli. Escherichia coli (26.6%) was the commonest isolate followed by S. aureus (13.1%) and coagulase-negative staphylococcus (CoNS) (13%). Methicillin resistance in S. aureus was found to be 36.6% and ESBL production was found in 162 (55.9%) Escherichia coli isolates and 22 (40.7%) Klebsiella spp. High level aminoglycoside resistance was observed in 15% enterococci. Gram-positive organisms showed maximum susceptibility to vancomycin and linezolid. Gram-negative bacilli especially members of Enterobacteriaceae were highly resistant to ampicillin, amoxicillin-clavulanate. Piperacillin-tazobactam combination and carbapenems showed best activity for gram-negative bacilli.

Conclusion: Continuous monitoring of antimicrobial susceptibility pattern in individual settings together with their judicious use is emphasized to minimize emergence of drug resistant bacteria.

Key words: *SSTIs, MRSA, ESBL, S aureus, Escherichia coli*

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INTRODUCTION

Skin and soft tissue infections (SSTIs) have variable etiology and clinical presentation and is commonly seen in both in ambulatory and hospital settings [1]. It may range from mild superficial infections such as cellulitis, furuncles, folliculitis which is often uncomplicated to deeper complicated SSTIs (cSSTIs) such as necrotizing fasciitis, surgical site infections and diabetic ulcers [2]. Deep seated complicated infections usually involve the subcutaneous tissues, fascia or the muscles which may progress rapidly and leads to septicemia and may increase the duration of hospital stay and cost of treatment [3]. It may also lead to complications that include osteomyelitis, endocarditis, gangrene, bacteremia and septicemia, especially in patients with comorbidities, such as diabetes, obesity, immune compromise, renal and hepatic diseases [4,5].

Although the etiology of SSTIs is uncertain, the

infection is commonly caused by Gram-positive pathogens, mainly *S. aureus* and beta-hemolytic streptococci in uncomplicated superficial infections [2,6]. Whereas, Gram-negative organisms are more frequently seen in healthcare-associated complicated SSTIs than community acquired complicated SSTIs [1,7]. Polymicrobial infections, especially mixed infections of Gram-positive and Gram-negative infections ranges from 10-24% and there is high risk of inappropriate empirical therapy in such cases [1,8].

Inadvertent use of antibiotics lead to emergence of antibiotic resistance among both the Gram-positive and Gram-negative organisms is posing a challenge to the treatment and outcome of SSTIs. Methicillin-resistant *S. aureus* (MRSA) is very commonly isolated agent of uncomplicated SSTIs and is seen more commonly in hospital settings especially in emergency departments as purulent SSTIs [9]. Risk factors associated with MRSA-SSTIs are previous history of MRSA infection, prolonged underlying disease, unhealed open wounds, elderly age group and long duration of hospital stay or frequent hospital visit [10]. Extended spectrum β- lactamases (ESBL) producing Gram-negative organisms have also been found to be commonly associated with cSSTIs [11].

Effective management of SSTIs by empirical therapy requires knowledge of potential pathogens and their antimicrobial susceptibility pattern. It is important to monitor the changing trends in bacterial infection and their antimicrobial susceptibility pattern to provide appropriate antimicrobial therapy for controlling infection, preventing morbidity and improve the quality of life. The present study was undertaken to determine the bacterial etiology and their antimicrobial susceptibility pattern of soft tissue infections among patients attending the tertiary care set up.

MATERIALS AND METHODS

The study was prospective observational cross-sectional study conducted in the department of microbiology. The pus samples received in the bacteriology laboratory from the patients attending the outpatient departments and admitted in inpatient departments with presentation of SSTIs were included in the study. Those patients with history of oral and/ or topical antibiotic usage in last 4 weeks were not included in the study. Demographic data (age, sex), clinical conditions data (history of antibiotic usage, fever and wound) and other relevant information about the participants was recorded from each participants in a structured questionnaire.

The received samples were processed immediately as per the standard laboratory protocol. All samples were inoculated in nutrient agar, MacConkey agar and 5% blood agar and incubated overnight at 37oC aerobically. Identification of the isolates were done on the basis of colonial morphology, Gram-staining and various biochemical tests such as catalase, coagulase, oxidase, indole, methyl-red, voges-proskauer, citrate utilization, sugar fermentation and decarboxylation test.

Antibiotic susceptibility testing was performed for the isolates obtained by Kirby-Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines. MRSA was detected by using cefoxitin (30µg) discs by Kirby-Bauer disc diffusion method as per CLSI 2018 guidelines. ESBL production in *Escherichia coli* and *Klebsiella spp*. was determined phenotypically by cephalosporin/clavulanate combination discs method using ceftazidime (30µg) disc alone and combination with clavulanate (10µg). Quality control was done by using non-ESBL producer Escherichia coli ATCC 25922 and ESBL producer *Klebsiella pneumoniae* ATCC 700603.

OBSERVATIONS AND RESULTS

Out of 1672 pus samples received, 1088 (65.1%) bacterial isolates were obtained. Of the positive culture, 736 (67.6%) were obtained from male patients and 352 (32.4%) were obtained from female patients. The growth was commonly isolated from the age group 25-35 years (31%) as shown in the Table 1.

Out of 1088 obtained bacterial isolates, 674 (61.9%) were Gram-negative bacteria, 398 (36.6%) were Gram-positive bacteria and 16 (1.6%) were gram positive bacilli. *Escherichia coli* (26.6%) was the commonest isolate followed by *S. aureus* (13.1%) and coagulase-negative *staphylococcus* (CoNS) (13%). The distribution of various bacterial isolates is depicted in Table 2.

Table 1: Distribution of bacterial isolates in various age groups.

Age group	Number of isolates (n=1088)
>15 years	152 (14%)
15-25 years	141 (13%)
25-35 years	342 (31%)
35-45 years	161 (15%)
45-55 years	127 (12%)
>55 years	165 (15%)

Note=Percentage in parenthesis represent out of total isolates obtained

Table 2: Distribution of isolates obtained from various pus samples.

Isolates	Number of isolates
Escherichia coli	290 (26.6%)
S. aureus	142 (13.1%)
CoNS	141 (13%)
Pseudomonas spp.	134 (12.3%)
Acinetobacter spp.	130 (12%)
Beta-hemolytic Streptococci	56 (5.1%)
Klebsiella spp.	54 (5%)
Citrobacter spp.	40 (3.7%)
Enterococcus spp.	40 (3.7%)
Proteus spp.	20 (1.8%)
Micrococcus spp.	19 (1.7%)
Diphtheroid spp.	16 (1.5%)
Enterobacter spp.	06 (0.5%)
Total	1088

Note= Percentage in parentheses represent out of total number of isolates, CoNS = Coagulase-negative *Staphylococcus*

Methicillin resistance was found in 52 (36.6%) *S. aureus* isolates, while none of the isolate showed inducible clindamycin resistance. High level aminoglycoside resistance was observed in 6 (15%) *enterococci* isolates. ESBL production was found in 162 (55.9%) *Escherichia coli* isolates and 22 (40.7%) *Klebsiella spp*.

Antibiotic susceptibility profile of Gram-positive isolates is depicted in Table 3. *S. aureus* isolates showed a higher resistance to penicillin, ampicillin and cotrimoxazole, whereas most of the isolates were susceptible to vancomycin (89.4%) and linezolid (97.9%). Similar findings were observed with beta-hemolytic *streptococcus* and *Enterococcus spp*. for vancomycin and linezolid. However, beta-hemolytic *streptococcus* were more susceptible to commonly used antibiotics as compared to *S. aureus* and *Enterococcus spp*.

Antibiotic susceptibility profile of Gram-negative isolates is demonstrated in Table 4. Gram-negative bacilli especially members of *Enterobacteriaceae* were highly resistant to ampicillin, amoxicillin-clavulanate, and 2nd generation oral cephalosporins. Resistance to fluoroquinolones was about 62%. Approximately 21.1% of them were resistant to fourth

Table 3: Antibiotic susceptibility profile of Gram-positive isolates.

Antibiotics	*S. aureus*	Beta-hemolytic Streptococcus	Enterococcus spp.
Penicillin	16(11.3%)	37(66%)	16(40%)
Ampicillin	28(19.7%)	29(51.2%)	19(47.5%)
Clindamycin	86(60.6%)	35(21.1%)	--
Erythromycin	91(63.4%)	31(21.8%)	--
Ciprofloxacin	73(51.4%)	50(89.2%)	23(57.5%)
Vancomycin	127(89.4%)	56(100%)	36(90%)
Linezolid	139(97.9%)	56(100%)	39(97.5%)
Cotrimoxazole	54(38.1%)	21(37.5%)	24(60%)
Cefoxitin	90(63.4%)	--	--
HLG	--	--	6(15%)
HLS	--	--	6(15%)

Note=Percentage in parenthesis represent out of total number of individual isolates, HLG=High-level gentamicin, HLS= High-level streptomycin

generation cephalosporin. *Pseudomonas spp*. showed maximum susceptibility to carbapenems followed by piperacillin–tazobactam. It was highly resistant to aztreonam. *Acinetobacter spp*. also showed a similar susceptibility pattern. Piperacillin-tazobactam combination and carbapenems showed best activity for gram-negative *bacilli*.

DISCUSSION

Skin and soft tissue infections (SSTIs) are among the common presentation in both outpatients and inpatients department. The major challenge to the treatment of SSTIs, especially cSSTIs, is the development of resistance to the commonly used oral and topical antibiotics. Methicillin resistance among *S. aureus* is very common and treatment of such cases relies mostly upon topical antibiotics such as mupirocin, which in turn increases the irrational use of these antibiotics leading to development of resistance to them. Although, *Escherichia coli* and *Pseudomonas spp*. are not as regularly found as Gram-positive organisms in SSTIs, still they constitute an important cause for HA-cSSTIs.

In this study the culture positivity rate was found to be 65.1% with most of cases were found to be mono-microbial which is consistent with the findings of studies conducted by Sah . et al (62%), Singh A (64.7%) and Gupta et al. (73.8%) [12-14]. The infections was more common in male compared to female (2.08:1) which is similar to the findings of Sharma et al. [15,16]. In this study, SSTIs is commonly seen in age group of 25-35 years of age group which comparable to various studies [16-18].

Table 4: Antibiotic susceptibility profile of Gram-negative isolates.

Antibiotics	EC	PS	AB	KL	CB	PR	EB
AMP	14(4.8%)	--	--	6(11.1%)	4(10%)	5(25%)	0
CZ	52(17.9%)	--	--	3(5.5%)	9(22.5%)	4(20%)	0
AMC	78(26.9%)	--	--	9(16.7%)	24(60%)	10(50%)	4(66.7%)
PIT	272(93.8%)	126(94.1%)	108(83.1%)	49(90.7%)	30(75%)	18(90%)	6(100%)
TCC	171(58.9%)	--	--	28(51.8%)	10(25%)	16(80%)	3(50%)
CPM	229(78.9%)	80(59.7%)	73(56.1%)	42(77.8%)	28(70%)	18(90%)	5(83.3%)
CTR	234(80.7%)	--	78(60%)	43(79.6%)	14(35%)	18(90%)	4(66.7%)
CAZ	--	54(40.3%)	75(57.7%)	--	--	--	--
DOR	280(96.5%)	130(97%)	122(93.8%)	50(92.6%)	35(87.5%)	19(95%)	6(100%)
IMP	274(94.5%)	130(97%)	124(95.4%)	49(90.7%)	38(95%)	19(95%)	6(100%)
MRP	278(95.8%)	104(74.6%)	101(77.7%)	51(94.4%)	28(70%)	19(95%)	6(100%)
G	261(90%)	74(55.2%)	69(53.1%)	49(90.7%)	39(97.5%)	19(95%)	4(66.7%)
TOB	265(91.4%)	92(68.6%)	111(85.4%)	50(92.6%)	39(97.5%)	19(95%)	4(66.7%)
AMK	275(94.8%)	66(49.2%)	79(60.7%)	50(92.6%)	38(95%)	18(90%)	4(66.7%)
CIP	173(59.7%)	81(60.4%)	73(56.1%)	36(66.7%)	30(75%)	16(80%)	5(83.3%)
LE	194(66.7%)	86(64.2%)	66(50.7%)	37(68.5%)	28(70%)	18(90%)	5(83.3%)
NIT	223(76.9%)	--	--	40(74.1%)	17(42.5%)	18(90%)	4(66.7%)
AT	--	6(4.5%)	--	--	--	--	--

Note: EC=*Escherichia coli*, PS=*Pseudomonas*, AB=*Acinetobacter*, KL=*Klebsiel*la, CB=*Citrobacter*, PR=*Proteus*, EB=*Enterobacter*, AMP= Ampicillin, CZ=Cefazolin, AMC=Amoxiclav, PIT=Piperacillin-tazobactam, TCC= Ticarcillin-clavulanate, CPM=Cefepime, CTR=Ceftriaxone, CAZ=Ceftazidime, DOR=Doripenem, IMP=Imipenem, MRP=Meropenem, G=Gentamicin, TOB=Tobramycin, AMK=Amikacin, CIP=Ciprofloxacin, LE=Levofloxacin, NIT=Nitrofurantoin, AT=Aztreonam

The commonest isolates found in this study is *Escherichia coli* (26.6%) followed by S. *aureus* (13.1%) and CoNS (13%) which is not consistent with the findings of various studies in which *S. aureus* was the commonest organisms in SSTIs [12-16]. This might be attributed to the late presentation of cases in the facility leading to complicated SSTIs.

Escherichia coli, the commonest isolate in the study showed high susceptibility to carbapenems, aminoglycosides and piperacillin-tazobactam; moderate susceptibility to cephalosporins, nitrofurantoin and fluoroquinolones; and least susceptibility to ampicillin and cefazolin. Similar susceptibility pattern was found in the study conducted by Sharma et al., Soumya et al., and Afroz et al. [15,16,19]. ESBL production was found in 55.9% isolates of *Escherichia coli* which is consistent with the findings of the studies conducted by Rao et al., and Fouzia et al., whereas Sharma et al., showed a higher rate of ESBL production [15,20,21]. Other members of Enterobacteriaceae showed similar pattern of susceptibility as shown by *Escherichia coli*.

Antibiotic sensitivity pattern showed that S. *aureus* isolates were least susceptible to penicillin (11.3%), ampicillin (19.7%) and cotrimoxazole (38.1%) and most susceptible to vancomycin (89.4%) and linezolid (97.9%). However, it was moderately susceptible to ciprofloxacin (51.4%), clindamycin (60.6%), and erythromycin (63.4%). Similar findings were observed in Sah et al. who showed 89.47% and 69.23% *S. aureus* isolates were resistant to amoxicillin and cotrimoxazole respectively [12]. Resistance to erythromycin (26.92%) and ciprofloxacin (29.41%) found in the study was also similar to the findings of our study. High susceptibility to vancomycin was found in studies conducted by Singh , Sah and Kamat et al. [12,13,22]. Methicillin resistance in *S. aureus* was found in 36.6% isolates which is similar to study conducted by Ioannou et al. (43.8%), Sharma et al. (40.25%), Singh et al. (28.57%) Gupta et al. (23.08%) and Waheed et al. (21.7%) [6,13,14,15,18].

Pseudomonas spp. and *Acinetobacter spp*. showed similar high sensitivity to carbapenems and piperacillin-tazobactam. Similar findings is also shown in the studies conducted by Kamat et al. Afroz et al. and Singh et al. [13,19,22]. However, susceptibility to fluoroquinolones, aminoglycosides and cephalosporins was relative less and it is consistent with the findings of studies conducted by Gupta et al. and Afroz et al. [14,19].

CONCLUSION

Continuous monitoring of antimicrobial susceptibility pattern in individual settings together with their judicious use is emphasized to minimize emergence of drug resistant bacteria.

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