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Bacteriological Profile and Antimicrobial Susceptibility Pattern of Skin and Soft Tissue Infections among Gram Negative Bacilli in a Tertiary Care Hospital of South India

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Abstract

Background-Skin & soft tissue infections (SSTIs) occurring either in community or hospital set up cause significant morbidity & warrant judicial antibacterial therapy.

Aims - To find the profile of bacteria causing skin and soft tissue infections& the antibiotic susceptibility pattern of the isolated bacteria.

Material and methods – It was a prospective study from April 2014 to September 2014. A total of 200 pus samples from SSTIs received in bacteriology laboratory were analysed.

Results – A total of 216 bacteria were isolated from 200 pus samples, with some samples showing growth of more than one bacteria. There were 92 (42.59%) gram positive cocci and 124 (57.40%) of gram negative bacilli. Among the gram negative bacilli *Pseudomonas aeruginosa* (21%) was the most common isolate followed by *Escherichia coli* (17.5%). ESBLwas found in (74.1%) of Gram negative bacilli. Gram negative bacilli were most susceptible to imipenem, amikacin and piperacillin/Tazobactam.

Conclusion : With the knowledge of the likely causative organism causing SSTIs and their resistance pattern the most suitable antibiotic can be started without waiting for antibiogram

Key Words - Skin & soft tissue infections, Extended spectrum beta lactamase, Antibiotic susceptibility pattern

INTRODUCTION

Skin and soft tissue infections(SSTIs) are a common type of infection that may contribute to longer hospital stays , increase the cost of medical care and play and important role in development of antimicrobial resistance. They are a common cause of morbidity in both community and hospital.¹

Common examples of SSTIs includes cellulitis, abscesses, impetigo, folliculitis, furuncle, carbuncle,necrotizing fasciitis , diabetic foot infections and surgical site infections .Superficial infections can be treated by oral antibiotics and topical care. Complicated SSTI may prove fatal and require hospitalization , intravenous antibiotic and or surgery .An SSTI is classified as complicated if the infection has spread to the deeper soft tissue, if surgical intervention is necessary or if the patient has comorbid conditions hindering treatment response (eg. Diabetes mellitus or human immune deficiency virus).^{1,2} SSTIs may be caused by a wide range of pathogens. *Staphylococcus aureus* is recovered from maximum number of SSTIs. Other organisms recovered included *Pseudomonas aeruginosa, Eschirichia coli, Enterococcus, Klebsiella and Enterobacter* species.²

Emergence of vancomycin resistant *S. aureus* (VRSA) and *Enterococcus* and ESBL producing gram negative pathogen have been observed which confer resistance to commonly used drugs and poses a significant problem both in community and hospital acquired infection in deciding empiric therapy. ³⁻⁶

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. . Since limited data are available concerning soft tissue infection, mortality rate and antibiotic susceptibility of Gram negative bacilli in our hospital settings , the present study will be taken to determine types, frequency of bacterial isolates and their antibiotic susceptibility pattern.

MATERIAL AND METHODS

The current study was a prospective study conducted from April 2014 to September 2014 which included a total of 216 culture positive samples . Patients of both sexes irrespective of age groups suffering from SSTIs attending or admitted in general surgery, orthopedics, dermatology, gastroenterology, gynaecology and intensive care units of Shri B.M. patil medical college were included in the study. **Inclusion criteria**: Single and mixed growth with Gram

negative bacilli were included in the study.

Exclusion criteria: Single and mixed growth which were not showing Gram negative bacilli were excluded from the study.

The lesions were cleaned with sterile normal saline. Special care was taken to avoid contamination by normal flora of skin or mucus surface, where possible pus was aspirated or exudates collected. The specimens were transported in sterile, leak-proof containers .The specimen were inoculated on nutrient agar, Mac-conkey agar and blood agar plates.Nutrient agar and Mac-conkey agar plates were incubated aerobically and blood agar plates were incubated in the presence of 5% CO₂ at 37°C overnight. The isolates were identified by gram staining, colony morphology and standard biochemical tests :catalase, slide and tube esculin hydrolysis ,bacitracin coagulase, oxidase, sensitivity test ,indole production, methyl red test, Voges-Proskauer test, citrate utilization (IMViC tests), H₂S production, ornithine decarboxylase, arginine hydrolase, lysine decarboxylase, urease, nitrate reduction test and sugar fermentation tests.⁷

Antimicrobial susceptibility testing

Antibiotic susceptibility tests were done on these isolates using Mueller-Hinton agar by standard Disc diffusion method according to CLSI guidelines. The following antibiotics were tested ampicillin (10µg), gentamicin (10µg), amikacin (30µg), piperacillin-tazobactam $(100/10\mu g),$ cefuroxime (30µg), cephalexin(30µg),ceftriaxone (30µg), cotrimoxazole(25µg), ceftazidime (30µg),tetracycline(30µg), ciprofloxacin (5µg) and imipenam (10µg). The diameter of zone of inhibition was recorded and interpreted as susceptible or resistance by criteria of CLSI.8

Detection of ESBL ⁹

Extended spectrum β lactamase (ESBL) was detected by phenotypic disc confirmatory test (PCT) using ceftazidime (30µg) and ceftazidime-clavulanic acid (30/10µg) discs. Organisms were deemed ESBL producing when zone of inhibition with ceftazidime- clavulanic acid disc was > 5mm as compared to ceftazidime disc alone.

STATISTICAL ANALYSIS

Data was analysed by SPSS 14 software and by proper diagrammatic presentation.

RESULTS

Of the 200 culture positive samples the number of bacterial isolates obtained were 216. 192 had single pathogen and 8 had two types of bacteria.

 TABLE 1
 DEMOGRAPHIC CHARACTERISTICS OF THE STUDY

| Age group in years | n% | | |
|--------------------|---------------|--|--|
| 0-10 | 3 (2.71.%) | | |
| 11-20 | 9 (8.3%) | | |
| 21-30 | 21 (19.44 %) | | |
| 31-40 | 25 (23.14%) | | |
| 41-50 | 16 (14.8%) | | |
| 51-60 | 21 (19.44 %) | | |
| >60 | 13 (12.03 %) | | |
| Gender | | | |
| Male | 80 (74.07 %) | | |
| Female | 28 (25.92 %) | | |

Most of the patients suffering from SSTIs were in 31-40 years of age group and majority were male patients. The demographic characteristics of the study is in(table 1). Most of the isolates in our study were from diabetic foot ulcer(33%) followed by abscess(29%) and cellulitis(12%) The distribution of cases in our study is given in (table 2).

TABLE 2DISTRIBUTION OF CASES IN SSTIS

| Diabetic foot ulcer | 36 (33.33 %) | | | |
|-------------------------|--------------|--|--|--|
| Abscess | 32 (29.62 %) | | | |
| Cellulitis | 13 (12.03 %) | | | |
| Wound infection | 10 (9.25 %) | | | |
| Necrotising fascitis | 8 (7.4 %) | | | |
| Surgical site infection | 6 (5.55 %) | | | |
| Carbuncle | 3 (2.7 %) | | | |

Out of the 216 bacterial isolates there were 92 (42.59%) Gram positive cocci and 124 (57.40%) of Gram negative bacilli (fig 2).The distribution of organisms is given in (table 3). *Pseudomonas aeruginosa* (21%) was the most common organism (35%) followed by and *Escherichia coli* (17.5%).

TABLE 3:ISOLATES OF SSTIS (N=124)

| Pathogen | N% |
|------------------------|------------|
| Pseudomonas aeruginosa | 46 (21%) |
| Escherichia coli | 38 (17.5%) |
| Klebsiella species | 20 (9.2%) |
| Citrobacter species | 16 (7.4%) |
| Acinetobacter species | 2 (0.9%) |
| Proteus vulgaris | 2 (0.9%) |

Antimicrobial susceptibility

Resistance pattern of GNB to various antibiotics is shown in table 4. Of 124gram negative isolates 92 (74.1%) were ESBL producing. Extended spectrum beta lactamase production was observed to be maximum in *Pseudomonas aeruginosa* (36.9%), followed by *Escherichia coli* (28.92%), *Klebsiella species* (15%) and Citrobacter species (15%), .ESBL production was found to be minimum in *Proteus and Acinetobacter* species (2.17%).Resistance of gram negative bacilli was minimum against imipenam (9.6%) followed by amikacin (51.6%) and piperacillin /tazobactam combination(54.81%).83.8% of pseudomonas aeruginosa were susceptible to piperacillin compared to other gram negative bacilli (63%).

| Antimicrobials in (µg) | P.aeruginosa (n=46) | <i>Ecoli</i> (n=38) | Klebsiella sp(n=20) | Citrobacter sp (n=16) | Acinetobacter sp(n=2) | Proteus vulgaris(n=2) |
|-------------------------------------|------------------------|------------------------|------------------------|--------------------------|--------------------------|--------------------------|
| Ampicillin (10) | 42 (91.30%) | 34 (89.4%) | 14 (70%) | 16 (100%) | 2 (100%) | 2 (100%) |
| Gentamicin(10) | 26 (56.52%) | 24 (63.1%) | 8 (40%) | 16 (100%) | 2 (100%) | 0 (nil) |
| Amikacin (30) | 22 (47.8%) | 24 (63.1%) | 8 (40%) | 10 (62.5%) | 0 (nil) | 0 (nil) |
| Piperacillin/ Tazobactam(100/10) | 14 (30.43%) | 24 (63.1%) | 12 (60%) | 16 (100%) | 2 (100%) | 0 (nil) |
| Cefuroxime (30) | 42 (91.30%) | 34 (89.4%) | 16 (80%) | 16 (100%) | 2 (100%) | 2 (100%) |
| Cephalexin (30) | 42 (91.30%) | 34 (89.4%) | 16 (80%) | 16 (100%) | 2 (100%) | 2 (100%) |
| Ceftriaxone (30) | 42 (91.30%) | 34 (89.4%) | 16 (80%) | 16 (100%) | 2 (100%) | 2 (100%) |
| Cotrimoxazole (25) | 38 (82.6%) | 28 (73.6%) | 10 (50%) | 12 (75%) | 2 (100%) | 0 (nil) |
| Ceftazidime (30) | 42 (91.30%) | 34 (89.4%) | 16 (80%) | 16 (100%) | 2 (100%) | 2 (100%) |
| Tetracycline (30) | 42 (91.30%) | 30 (78.9%) | 16(80%) | 8 (50%) | 2 (100%) | 0 (nil) |
| Ciprofloxacin (5) | 30 (65.21%) | 28(73.6%) | 16 (80%) | 14 (87.5%) | 0 (nil) | 0 (nil) |
| Imipenam (10) | 4 (8.6%) | 1 (2.63%) | 0 (nil) | 4 (25%) | 2 (100%) | 0 (nil) |
| Piperacillin (100) | 20 (43.4%) | 22 (57.89%) | 10 (50%) | 10 (62.5%) | 2 (100%) | 2 (100%) |

TABLE 4: ANTIMICROBIAL RESISTANCE PATTERN OF GRAM NEGATIVE ORGANISMS FOUND IN SSTIS

DISCUSSION

Pseudomonas aeruginosa and Escherichia coli were the predominant organisms among the GNB in the present study which is similar to studies in India^{1,9} and outside ^{10,11} were they were the most frequently isolated organism among the predominant five organisms isolated from skin and soft tissue infections in patient admitted to the hospital. Majority of the cases in present study were males (74.07%) and the most common age group was 31 - 40 years (23.14%) which is similar to the study conducted by Malhotra *et al* where the percentage of male was (67.21%)and the major age group was 31-40 years.¹² Maximum resistance of Gram negative organisms was seen against cephalosporins like cefuroxime, cephalexin, ceftriaxone and ceftazidime (90.32%) followed by ampicillin (88.7%) and tetracycline (79.03%) which is in contrast with the study conducted by Alireza Sharif where the resistance of Gram negative organisms to cephalexin was (35%).¹³ Resistance of Gram negative organisms was minimum against imipenem (9.67%), followed by amikacin (51.61%) and Piperacillin - tazobactam (54.83%) which is similar to other studies .14,15,16

ESBL production was seen in (74.1%) of gram negative isolates which was similar to the studies conducted in AIIMS Delhi $(66.75\%)^1$ and by mathur P $(68\%)^{17}$.ESBL producing isolates were resistant to other antibiotics like fluoroquinolones & cephalosporins ^{1,17}. This was also seen in our study. In our study Imipenam, amikacin & piperacillin/tazobactam were the most effective drugs among ESBL producing GNB(85%) compared to the study were susceptible^{1,18} were (90%) of ESBL isolates Currently there is paucity of data on prevalence of ESBL in skin and soft tissue infection. The prevalence of ESBL among members of enterobacteriaceae constitutes a serious threat to the current beta lactam therapy leading to treatment failure.

CONCLUSION

In this present study the most common isolate in skin and soft tissue infection among Gram negative bacilli is *Pseudomonas aeruginosa* followed by *E. coli*. Most of *the* Gram negative bacilli were ESBL producing. Imipenam, Amikacin,& piperacillin –tazobactam (Beta lacta – beta lactam inhibitor combination) would me most useful against GNB. With this knowledge of likely causative organisms causing SSTIs and their sensitivity pattern, the most suitable antibiotic can be started without waiting for the result. This would help in avoiding unnecessary medication with ineffective antibiotics and prevent development drug resistance.

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