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Research Article

Antibiotic resistance pattern of bacterial isolates from skin and soft tissue infections

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ABSTRACT

Background: Skin and soft tissue infections (SSTIs) are commonly encountered in clinical practice. The spectrum of the causative bacterial agents changes constantly, and so does their antibiogram. Hence, this study was carried out to find the etiological bacterial agents and their antibiotic resistance patterns in cases of SSTIs.
Methods: A pus sample was collected aseptically from 328 cases of skin and soft tissue infections attending OPD of a tertiary care hospital and was investigated for antibiotic resistance pattern of isolated organism.
Results: *Staphylococcus aureus* was the commonest pathogen. It showed maximum resistance against penicillin (97.70%) and 100% sensitivity to teicoplanin, linezolid, gentamicin and vancomycin. 75.86% of the isolated *S.aureus* strains were methicillin sensitive and 24.14% were methicillin resistant. *Pseudomonas aeruginosa* was the second most frequent isolate. It showed maximum resistance to aztreonam (64.3%), followed by piperacillin, ceftazidime (57.1%) and was completely sensitive to imipenem.
Conclusions: *Staphylococcus aureus* exhibited high resistance to commonly prescribed antibiotics like β-lactams, fluroquinolones and fusidic acid. Hence, it is recommended to base the treatment upon culture and sensitivity report.

Keywords: Antibiotic resistance, *Staphylococcus aureus*, Skin and soft tissue infections

INTRODUCTION

Skin and soft tissue infections (SSTIs) can be defined as 'an inflammatory microbial invasion of the epidermis, dermis and subcutaneous tissues'.[1] It is quite commonly encountered in clinical practice, encompassing a wide variety of presentations ranging from simple impetigo to life-threatening necrotizing fasciitis. The SSTIs are usually caused by Gram positive bacteria like *Staphylococcus aureus* and group A β-hemolytic Streptococcus and less commonly, by Gram negative organisms like *Escherichia coli, Klebsiella species, Pseudomonas aeruginosa*, Proteus species, etc.[2]

Appropriate topical/systemic antibacterial therapy forms an important component in the management of these lesions. But, the increasing resistance to the antibiotics prevailing in microorganisms is posing a big problem to the clinicians.[2] Many cases do not respond to same antibiotics which were previously effective. Also, the antibiotic sensitivity pattern shows temporal and geographic variations. Hence, there is a constant need to monitor the changing trends of causative bacterial agents and their antibiogram.

Considering these facts, the present study was carried out to find out the etiological bacterial agents and their antibiotic resistance patterns in cases of SSTIs attending dermatology and surgery OPD in a tertiary care hospital.

METHODS

A cross-sectional analytical study was conducted at Department of Microbiology, Alluri Sitarama Raju Academy of Medical Sciences Hospital, Eluru, Andhra Pradesh, India from July 2010 to August 2011. Wound swabs from 328 patients presenting with SSTIs attending the dermatology and surgery outpatient departments were collected for bacteriological examination. Patients on antibiotic therapy in the past two weeks were excluded from the study. Pus sample was collected aseptically with the help of two sterile swabs; one was used for Gram stain and the other for culture on blood agar and Mac Conkey agar plates. From subcutaneous abscess cases, pus was aspirated in stopper syringes. Inoculated plates were incubated at 37^0C for 24 hours. Plates showing no growth during the first 24 hours were further incubated for another 24 hours. Preliminary identification of bacteria was done on the basis of colony characteristics. Subsequently, Gram staining, motility and standard biochemical tests were performed. Biochemical tests employed were slide & tube coagulase test, oxidase, catalase, nitrate and indole production tests, methyl red test, Voges Proskauer test, citrate utilization test, urea hydrolysis test, sugar fermentation and H2S production on TSI medium.

Antibiotic sensitivity

Antibiotic sensitivity testing of all isolates was done on Mueller Hinton agar plates by Kirby-Bauer disc diffusion method. Results of antimicrobial susceptibility test were interpreted as per CLSI guidelines 2008.

Statistical analysis

The collected data was analysed with the aid of the Statistical Package for Social Sciences Version 10 software. A p-value <0.05 was considered as statistically significant.

RESULTS

A total of 328 samples were collected from patients with clinical evidence of SSTIs, out of which pyoderma constituted 176 (53.66 %) cases and soft tissue infections comprised of 152 (46.34 %) cases. Distribution of these cases is depicted in Table 1. Of these, 90.85 % cases showed culture positivity and 9.15 % cases showed no growth.

Out of 176 pyoderma cases subjected to aerobic bacterial culture, 160 cases showed bacterial growth. 156 (97.5%) samples yielded single isolate (140 *Staphylococci* and 16 β-hemolytic Streptococci) and 4 (2.5 %) cases had dual growth of *Staphylococci* and β-hemolytic Streptococci. Amongst the 152 soft tissue infection cases, 138 (90.79 %) cases yielded bacterial growth and the remaining 14 cases didn't show any growth. 118 (85.50%) samples yielded single pathogen, among which *Staphylococci* was isolated from 82 samples, followed by *Klebsiella* spp (16 cases), *Pseudomonas aeruginosa* (12 cases), *Escherichia coli* (6 cases) and *Proteus* spp. (2 cases). 20 (14.50%) cases yielded dual bacterial growth consisting of *Staphylococci* + *Pseudomonas aeruginosa* (14 cases) followed by *Staphylococci* + *Escherichia coli* (4 cases) and *Escherichia coli* + *Pseudomonas aeruginosa* (2 cases).

Table 1: Distribution of cases of pyoderma and soft tissue infections.

Type of Lesion			Number of cases (%)
Pyoderma (n= 176)	Impetigo	56	176 (53.66%)
	Folliculitis	32	
	Furuncle	16	
	Carbuncle	12	
	Dermatitis	32	
	Infected Scabie	28	
Soft tissue Infections (n=152)	Wound infection	90	152(46.34%)
	Subcutaneous Abscess	62	
Total		328	328(100%)

Table 2: Antibiotic resistance pattern (in percentage*) noted in gram-positive isolates.

Antibiotic tested	*S.aureus*	CNS	*β hemolytic Streptococci*
Penicillin (10 units)	97.70	100	NT
Cefoxitin (30 µg)	24.14	32.85	NT
Moxifloxacin (5 µg)	43.68	54.28	NT
Levofloxacin (5 µg)	29.88	34.30	30
Trimethoprim-sulfamethoxazole (1.25+23.75 µg)	28.73	40.00	NT
Fusidic acid (30 µg)	24.14	30.00	NT
Framycetin (100 µg)	22.98	32.80	NT
Pristinomycin (15 µg)	19.54	22.85	NT
Mupirocin (5 µg)	13.79	15.71	NT
Erythromycin (15 µg)	10.34	15.71	50
Rifampicin (5 µg)	6.89	20.00	NT
Clindamycin (2 µg)	4.59	7.14	10
Gentamicin (10 µg)	0	0	30
Linezolid (30 µg)	0	0	0
Teicoplanin (30 µg)	0	0	NT
Vancomycin (30 µg)	0	0	0
Cephotaxime (30 µg)	NT	NT	0
Ampicillin (10 µg)	NT	NT	0

Key: *denotes the percentage of isolates that are resistant to particular antibiotic; CNS: Coagulase negative Staphylococcus; NT**:** not tested.

Staphylococci was the commonest bacterial isolate for both pyoderma and soft tissue infections. The association was found to be statistically significant (Chi-square test

value = 65.28, p<0.001). Among the total 244 isolates (144 from pyoderma & 100 from soft tissue infection) of staphylococci obtained, 174 (71.31%) isolates were of *Staphylococcus aureus* and 70 28.69% isolates were that of coagulase negative Staphylococci (CNS).

Antimicrobial susceptibility

Antimicrobial susceptibility testing was carried out on all the isolates. The antibiotic resistance pattern of gram positive & Gram negative organisms is illustrated in Table 2 and 3.

Table 3: Antibiotic resistance pattern (in percentage*) noted in gram-negative isolates.

Antibiotic tested	*Escherichia coli*	*Klebsiella spp*	*Proteus spp*	*Pseudomonas aeruginosa*
Ampicillin (10 µg)	100	100	100	Nt
Cephalothin (30 µg)	100	100	100	Nt
Piperacillin (100 µg)	50	50	100	57.1
Cephotaxim (30 µg)	33.33	37.5	0	Nt
Gentamicin (10 µg)	33.33	12.5	0	14.3
Levofloxacin (5 µg)	33.33	0	0	Nt
Amikacin (30 µg)	16.66	12.5	0	14.3
Imipenem (10 µg)	0	12.5	0	0
Aztreonam (30 µg)	NT	NT	NT	64.3
Ceftazidime (30 µg)	NT	NT	NT	57.1
Cefepime (30 µg)	NT	NT	NT	7.1

Key: *Denotes the percentage of isolates that are resistant to particular antibiotic; NT**:** not tested.

Gram positive organisms

Staphylococcus aureus isolates showed maximum resistance against penicillin 97.70% followed by moxifloxacin 43.68%. 100% Sensitivity was observed with teicoplanin, linezolid, gentamicin and vancomycin. Also noteworthy is the fact that, of the 174 *Staphylococcus aureus* isolates, 132 (75.86%) were methicillin sensitive and 42 (24.14%) were methicillin resistant.

β-hemolytic streptococci strains were most resistant to erythromycin (50%) followed by gentamicin (30%), levofloxacin (30%) and clindamycin (10%) whereas complete sensitivity was noted for cefotaxime, vancomycin, linezolid and ampicillin.

Gram negative organisms

All *Escherichia coli* isolates were completely resistant to ampicillin and cephalothin and completely sensitive to imipenem. A similar 100% sensitivity pattern with imipenem was also seen with Proteus and Pseudomonas isolates. All strains of *Klebsiella spp* were sensitive to levofloxacin and were maximally resistant to ampicillin and cephalothin.

DISCUSSION

Out of 328 cases of SSTIs studied, impetigo was the major group in pyoderma whereas soft tissue infections were dominated by cases of wound infections. The similar finding of impetigo being the largest group amongst cases of pyoderma were observed by Ghadage et al, Ahmed et al, Baslas et al and Mathew et al.[3-6] In case of soft tissue infections, Buck JM et al observed more number of abscess cases than wound infection as opposed to our findings.[7]

Culture positivity of 90.85% achieved in our study is on par with many other studies from different parts of the country such as Ramana et al, Patil et al, Ghadage et al, and Baslas et al who reported culture positivity of 93.6%, 83.7%, 95% and 85.08% respectively.[2,3,5,8]

Staphylococcus aureus was the commonest isolate in our study. Earlier, many other investigators Mathew et al,[6] Baslas et al, Ahmed et al, Misra et al, Ghadage et al, Sugeng et al, Fatani et al, Mohanty et al, Abdallah et al[13] have similarly found *Staphylococcus aureus* to be the major isolate.[3-6,9-13] Recently, Singh et al and Malik et al too have found *Staphylococcus aureus* to be the causative agent of pyodermas in Rajasthan.[14,15]

Way back in 1968, Dillon HC reported *Streptococcus* to be the leading etiological agent of impetigo.[16] But, this trend has changed over the past few years as, we and others have found *S. aureus* to be the commonest causative agent of impetigo.[3,5,14,17,18]

The study conducted by Sanjay KR et al, showed Gram negative bacilli (72.45%) as the predominant isolates in cases of postoperative wound infection, thereby indicating that the causative organism may vary depending on the clinical setting and compounding factors.[19] Another study has also shown similar results.[20]

Amongst gram negative bacilli, *Pseudomonas aeruginosa* was the predominant isolate in this study as opposed to other studies wherein *Escherichia coli* is quoted as the leading organism.[12,14,19,20]

Antimicrobial resistance pattern of gram positive cocci isolated from SSTIs

In the present study, *Staphylococcus aureus* showed maximum resistance to penicillin 97.70% and all isolates were sensitive to vancomycin, teicoplanin, linezolid and gentamicin. This is in correlation with the study of Thind et al, where *Staphylococcus aureus* showed 100% resistance to penicillin and 100% sensitivity to vancomycin, teicoplanin and linezolid.[21] Ramana et al, Nagaraju et al, Patil et al, Misra et al and Singh et al observed a similar high resistance of S. aureus to penicillin.[2,8,9,14,22] Majority of β-hemolytic streptococci were resistant to erythromycin 50% in the present study. Ghadage et al, observed a similar pattern of resistance of β-hemolytic streptococci to erythromycin 48%.[3]

Antimicrobial resistance pattern of gram negative bacilli isolated from SSTIs

In our study, *Escherichia coli and Klebsiella spp*. were resistant to ampicillin and cephalothin followed by piperacillin 50%. All *Escherichia coli* and *Klebsiella* strains were completely sensitive to imipenem and levofloxacin, respectively. Only two *Proteus* species were isolated which exhibited resistance to ampicillin, cephalothin and piperacillin and susceptibility to cefotaxime, gentamicin, levofloxacin, amikacin and imipenem. *Pseudomonas* isolates showed maximum resistance to aztreonam 64.3%, followed by piperacillin, ceftazidime 57.1% and were completely sensitive to imipenem. The resistance patterns of gram negative bacilli in the present study were somewhat similar to the findings of Misra et al and Sanjay KR et al.[9,19]

CONCLUSION

The most common isolate from SSTIs in our study was *Staphylococcus aureus* which exhibited high resistance to β-lactams, fluroquinolones and fusidic acid that are commonly used antibiotics in outdoor healthcare settings. Hence, it is recommended to base the treatment upon culture and sensitivity report rather than injudicious use of antibiotics, even in outpatients.

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