Bacteriological profile and antimicrobial susceptibility pattern of pyogenic wound infections at a tertiary care hospital

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Abstract: *Introduction:* Infection of the skin and soft tissue due to injury, surgery, or burns may result in the formation of allexudates. Dead leucocytes, cellular debris and necrotic tissue are responsible for formation of exudates. Relentless exposure to devitalized tissue associated with a slow-healing chronic wound is likely to promote the colonisation and establishment of a wide range of bacteria. Mixed populations of both aerobic and anaerobic microorganisms are accountable for most acute and chronic wound infections. Therefore this study was carried out to investigate the aerobic bacterial isolates responsible for pyogenic wound infections and their antimicrobial susceptibility pattern for clinical management. *Material and Methods:* Pus samples were collected with sterile disposable cotton swabs and pus aspirates in syringes from suspected patients of pyogenic wound infections. They were processed using standard microbiological techniques and identification of isolate from positive cultures was done using conventional biochemical test. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines. *Results:* In our study out of 288 samples, 192(66.66%) were culture positive isolates and 96 (33.33%) were culture negative isolates. Out of 192 culture positive isolates, 76(39.58%) were Gram positive isolates and 116(60.41%) were Gram negative isolates. Staphylococcus aureus was most commonly isolated among all culture positive isolates. *Conclusion:* This study gives an outline of antibiotic susceptibility pattern of clinical isolates causing pyogenic wound infections which will help in empirical treatment of patients.
Keywords: Multidrug Resistant, Pyogenic Infections, Bacteriological Profile.

Introduction

Sepsis is frequently caused by pyogenic infections [1]. Pyogenic infection is marked by significant local inflammation, which is generally accompanied by pus development. Pyogenic infections may be endogenous or exogenous. Microbial pathogens cause human skin and soft tissue infections during or after trauma, burn injuries and surgical procedures [2].

The loss of skin integrity due to a variety of circumstances would create an ideal habitat for microbial colonisation and proliferation.Immune cells are brought into the area to attack bacteria as part of the body's defence process. Aggregation of these cells eventually results in pus, a thick white yellowish liquid [3]. When virulence factors released by one or more bacteria in a wound out compete the host's natural immune system, the micro organism invade and spread in viable tissue, triggering a sequence of local and systemic host reactions. A purulent discharge or painful spreading erythema around a lesion are typical local responses to cellulites [4]. The type, site, size, and depth of the wound, the level of blood perfusion to the wound, the host's general health and immune status, the microbial load, and virulence expressed by the types of micro organisms involved are all likely to play a role in the progression of a wound to an infected state. Most acute and chronic wound infections involve mixed populations of both aerobic and anaerobic micro organisms.

Complications arising from cutaneous and soft tissue infections with *S. aureus* are serious clinical problems owing to the high occurrence of these infections and the widespread evolution of antibiotic-resistant bacterial strains [5]. Gram positive cocci, such as Staphylococcus aureus and Staphylococcus epidermidis, are the most prevalent organisms found in pyogenic wound infections, followed by gram negative bacilli, such as Klebsiella spp., Pseudomonas spp., Escherichia coli., Proteus spp., Citrobacter spp., Acinetobacter spp. respectively [6].

Effective wound infection treatment requires a thorough understanding of the causal pathogen, the infectious process pathophysiology, and pharmacology of the therapeutic agents. Multidrug-resistant organisms continue to be a major source of hospital-acquired infections and offer therapeutic difficulties. Controlling the infection, minimising morbidity, and improving the quality of life require early diagnosis and prompt initiation of antimicrobial therapy.Empiric antimicrobial therapy results into widespread antibiotic resistance. To overcome these challenges and improve the outcome of major infections in hospital settings, it is necessary to monitor resistance patterns in the hospital [7].

Multidrug-resistant Gram-negative bacterial strains such as Acinetobacterbaumannii, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Gram-positive methicillin-resistant Staphylococcus aureus (MRSA) have become increasingly associated with pyogenic infections in hospital settings over the last few decades due to widespread antibiotic overuse and inadequate dose regimens [8]. Resistant bacteria infections are more likely to lengthen hospital stay, increase the risk of death, and need the administration of more expensive antibiotics. Furthermore, extremely virulent strains and the ability to adapt fast to changing environments exacerbate the problem and raise concerns. Hence, this study was carried out to investigate the bacterial isolates responsible for pyogenic wound infections and their antimicrobial susceptibility pattern.

Material and Methods

Study Design: This Study was carried out in the Department of Microbiology at tertiary care center, Chiplun, Maharashtra during the period of January 2017 to July 2019. Clinical specimens such as pus, wound aspirate and wound swab were collected aseptically from suspected patients with pyogenic wound infections and processed in the microbiology laboratory with minimal delay.

A total of 288 samples from patients of wound infections were collected and processed according to the standard laboratory guidelines. Two pus swabs were collected; one for the direct microscopy and the other for culture. The pus specimens were cultured onto the MacConkey agar and Blood agar plates (Hi Media) and incubated aerobically for 24-48 hours overnight at 37^0C. The culture plates were examined for bacterial growth and identified using standard microbiological techniques. The antibiotic susceptibility testing of all isolates was then performed by Kirby Bauer's disc diffusion method on Mueller Hinton agar. Antibiotic sensitivity results were interpreted as per CLSI guidelines.

Aims & Objective:

1. To identify aerobic bacterial isolates causing pyogenic wound infection using standard microbiological procedures.
2. To carry out antimicrobial susceptibility testing of these isolates by using the Kirby-Bauer Disc diffusion method.

Inclusion Criteria: All pus samples /wound swab collected aseptically were included in the study.

Exclusion criteria: Pus samples received in unsterile containers were rejected. Clinical samples other than pus / wound swab were excluded in the study.

Results

In this study total of 288 samples from patients of pyogenic wound infections were collected and processed according to the standard laboratory guidelines. Out of 288 samples, 192 (66.66%) were culture positive isolates and 96 (33.33%) were culture negative isolates (Table 1). Out of 192 culture positive isolates, 76(39.58%) were Gram

positive isolates and 116(60.41%) were Gram negative isolates.

Table-1: Culture Positivity of Pyogenic wound infections

Culture	Frequency	Percentage
Growth	192	66.66%
No growth	96	33.33%
Total	288	99.99%

Among the 192 culture positive samples, Staphylococcus aureus was predominant bacterial isolates 66 (34.37 %) followed by *Escherichia coli* 57 (29.68%), *Pseudomonas aeruginosa* 28(14.58%), *Klebsiella species* 25(13.02%), *Enterococcus species* 6 (3.12%), *Streptococcus pyogenes* 4 (2.08%), *Proteus species* was 4 (2.08 %) and *Enterobacter species* was 2(1.04%) (Table 2).

Table-2: Distribution of aerobic bacterial isolates in Pyogenic wound infections

| Sr. No.	Organism	Frequency	%
1	Staphylococcus Aureus	66	34.37%
2	Escherichia coli	57	29.68%
3	Pseudomonas aeruginosa	28	14.58%
4	Klebsiella species	25	13.02%
5	Enterococcus species	6	3.12%
6	Streptococcus pyogenes	4	2.08%
7	Proteus species	4	2.08%
8	Enterobacter species	2	1.04%

Discussion

In our study out of 288 samples, 192(66.66%) were culture positive isolates and 96(33.33%) were culture negative isolates (Table 1). Among the 192 culture positive samples, Staphylococcus aureus was predominant bacterial isolates 66 (34.37 %) followed by *Escherichia coli* 57 (29.68%), *Pseudomonas aeruginosa* 28(14.58%), *Klebsiella species* 25(13.02%), *Enterococcus species* 6 (3.12%), *Streptococcus pyogenes* 4 (2.08%), *Proteus species* was 4 (2.08 %) and *Enterobacter species* was 2(1.04%) (Table 2).

Out of 192 culture positive isolates, 76(39.58%) were Gram positive isolates and 116(60.41%) were Gram negative isolates (Table 3). There was a preponderance of Gram negative organisms observed in our study. This was in accordance with study done by Nithya et al [9].

Table-3: Distribution of Gram positive and Gram negative isolates in Pyogenic wound infections

Sr. No.	Isolates	Frequency	%
1.	Gram positive isolates	76	39.58%
2.	Gram negative isolates	116	60.41%

In the present study *Staphylococcus aureus* was most commonly isolated among the Gram positive cocci. Similar to the present study result Mantravadi et al [10] have revealed that *S. aureus* is the most commonly isolated pathogen (37.2%) in pus samples, which is in agreement with the studies by Rao et al [11], Tiwari et al [12]. However, our results are not in agreement with Agnihotri et al [13]. They found S. aureus to be the second most common pathogen after *Pseudomonas species*.

**Graph-1:** Antimicrobial susceptibility pattern of Gram positive organisms in Pyogenic infections
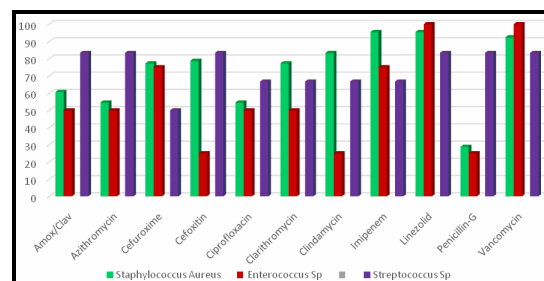
Escherichia coli was most commonly isolated among Gram negative organisms followed by *P.aeruginosa*, *Klebsiella sp*, *Proteus sp* and *Enterobacter species*. Similarly, Shekokar D. et al [14] found that among gram negative organism *Escherichia coli* (34.69%) was predominant followed by *Klebsiella spp*. (28.57%) and *Pseudomonas spp*. (15.30%). Our findings did not correlate with Zhang et al who reported predominance of *E.coli* followed by *S.aureus*, *K.pneumoniae* and

P.aeruginosa from pus samples [15]. In this study *Staphylococcus aureus* were 95.46% sensitive to Imipenem, 92.43% sensitive to Vancomycin, 83.34% sensitive to Clindamycin, 77.28% sensitive to Cefuroxime, 78.79% sensitive to Cefoxitin and 60.61% sensitive to Amoxycillin and Clavulonic acid (Graph1).

Enterococcus species were found sensitive to Linezolid (100%), Vancomycin (100%) and Cefuroxime (75%). *Streptococcus pyogenes* were 83.34% sensitive to Amoxycillin & Clavulonic acid, 66.67% to Clarithromycin and 66.67% Ciprofloxacin. (Graph 1) *E. coli* isolates were susceptible to species isolated were 96.5% sensitive to Netilmycin, 89.48% sensitive to Meropenem, 89.48% sensitive to amikacin, 82.46% sensitive to Cefaperazone & sulbactum, 78.95% sensitive to Gentamycin, 73.69% sensitive to Ceftazidime clavulanic acid and 61.41% sensitive to Ciprofloxacin. (Graph2).

Graph-2: Antimicrobial susceptibility pattern of gram negative isolates in Pyogenic infections
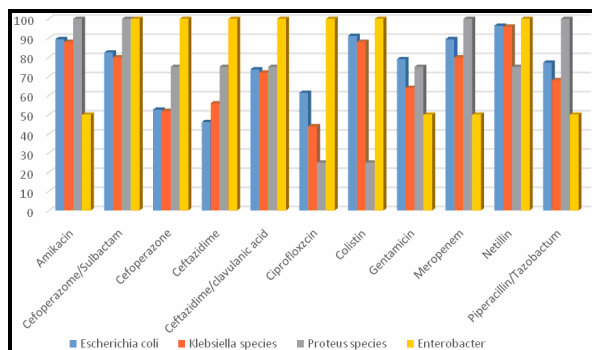
Pseudomonas aeruginosa were 96.42% sensitive to Polymyxin B, 92.85% sensitive to Meropenem, 92.85% sensitive to Cefoperazone & sulbactum, 89.28% sensitive to Piperacillin& tazobactum, 89.28% sensitive to Ceftazidime &clavulanic acid,78.57% sensitive to Ciprofloxacin and 75% sensitive to Cefoperazone. *Klebsiella species* were 96% sensitive to Netilmycin, 88% ,88% sensitive to amikacin, 80% sensitive to Meropenem, , 80% sensitive to Cefaperazone & sulbactum, 72% sensitive to Ceftazidime & clavulanic acid and 64% sensitive to Gentamycin**.** Proteus species isolates were susceptible to species isolated were 100% sensitive to amikacin,

75% sensitive to Ceftazidime and 75% sensitive to Ceftazidime clavulanic acid (Graph2).

Enterobacter species was 100% sensitive to Ceftazidime, 100% to Cefaperazone & sulbactum, and 50% sensitive to Amikacin. *E. coli* showed maximum resistance against Cefoperazone (47.36%) and Ceftazidime (53.85%). *P.aeruginosa* showed maximum resistance against Cefepime (53.48%) and Ceftazidime(46.43%) (Graph2). High antibiotic resistance was seen by S. aureus to penicillin. Highest sensitivity was shown by high-end drugs such as linezolid (95.46%) and vancomycin (92.43%).

High resistance was seen by gram-negative bacteria to third generation cephalosporins and Amikacin among the aminoglycosides showed good sensitivity. Similar studies by Taiwo et al [16] and Basu et al [17] corroborated our findings.

Conclusion

Despite breakthroughs in microbiological techniques, antibiotics, and surgical therapy, pyrogenic infections are still common in developing countries, and treatment remains a significant issue. It is vital to identify and treat the source of inflammation in order to ensure proper and effective treatment.

In the present study *Staphylococcus aureus* was most commonly isolated among the Gram positive organisms and *E. coli* among the Gram negative organisms. High resistance was seen by gram-negative bacteria to third generation cephalosporins. There fore, appropriate antibiotic selection based on antibiotic sensitivity data, as well as avoiding overuse, frequent misuse, and inadequate dosages, will minimise the emergence of drug resistant strains in the future, allowing for successful treatment of various clinical problems. As a result of our research, clinicians will be able to judiciously use antibiotics, which will not only result in improved treatment, but will also assist to prevent the emergence of drug resistance.

Conflicts of interest: There are no conflicts of interest.

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