Bacteriological Study of Wound Infections and Their Antibiogram Pattern at A Tertiary Care Hospital in Suburban Area of Hyderabad

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ABSTRACT

Background: Wound infections can be caused through two major sources: exogenous and endogenous bacteria. The probability of wound infection largely depends on the patient’s systemic host defenses, local wound conditions and microbial burden. Despite modern surgical techniques and the use of antibiotic prophylaxis, Surgical Site Infection (SSI) is one of the most common complications encountered in surgery. SSI places a significant burden on both the patient and health system. SSI is thus a major cause of morbidity, prolonged hospital stays and increased health costs.

Methods: Two wound swabs were collected from the wound and from a drop of aspirate, smear was made on clean glass slide and Gram staining was done for direct microscopic examination under oil immersion 100X objective to know various morphological types of bacteria and presence or absence of inflammatory cells. Second swab/drop of aspirate was used for culture by inoculating it on routine media like Blood Agar, Nutrient Agar and MacConkey’s agar, incubated at 37° C for 24 hrs. aerobically.

Result: Out of 108 pus samples, 101(88.5%) were culture positive for bacterial growth and no growth was observed in 7(11.4%) cases. Out of 101 bacterial culture positive cases, 96 were nonbacterial and 5 were poly bacterial. Out of 96 bacterial isolates; S. aureus (38/36.6%) was the commonest followed by P. aeruginosa (26/24.7%)

Conclusion: The study concludes that variety of aerobic bacteria is responsible for wound infection with predominance of Staphylococcus aureus followed by Pseudomonas aeruginosa

Keywords: Staphylococcus Aureus, Pseudomonas Aeruginosa, Nonbacterial

Introduction

Infection is defined as invasion and multiplication of microorganisms in the body tissues, which may be clinically in apparent or result in local cellular injury because of competitive metabolism, toxins, intra-cellular replication or antigen antibody response [1]. This series of events lead to progressive tissue destruction and eventual host demise if left unchecked. The infection process begins with a disruption of the host mechanical barriers to microorganisms, the availability of microorganisms and colonization.[2]

Wound infections can be caused through two major sources: exogenous and endogenous bacteria. The probability of wound infection largely depends on the patients systemic host defenses, local wound conditions and microbial burden [3, 4]. Despite modern surgical techniques and the use of antibiotic prophylaxis, Surgical Site Infection (SSI) is one of the most common complications encountered in surgery [5]. SSI places a significant burden on both the patient and health system. SSI is thus a major cause of morbidity, prolonged hospital stay and increased health costs [6].

Skin and soft tissue infections (SSTIs) may also contribute to longer hospital stays increase the cost of medical care and play an important role in development of antimicrobial drug resistance. Common examples of SSTIs includes cellulitis, abscesses, impetigo, folliculitis, furuncle, carbuncle, necrotizing fascitis, diabetic foot infections and surgical site infections. Complicated SSI may prove fatal and require hospitalization, intravenous antibiotics or surgery. An SSI is classified as complicated if the infection has spread to the deeper soft tissue, if surgical intervention is necessary or if the patient has co-morbid conditions. Hence, this study could play a significant role in the early recognition of the problem and hence, there is need for early intervention for better management of wound infections.

AIM & OBJECTIVES 1. To isolate and identity the aerobic bacterial pathogens from wound infections. 2.
To determine the antibiotics susceptibility pattern of the isolated pathogens with special reference to Methicillin resistant Staphylococcus aureus (M R S A) and Extended spectrum of β-lactamases (E S B L). 

3. To find out the incidence of aerobic bacterial pathogens in infected wounds.

Material and Methods

Two wound swabs were collected from the wound and from a drop of aspirate, smear was made on clean glass slide and Gram staining was done for direct microscopic examination under oil immersion 100X objective to know various morphological types of bacteria and presence or absence of inflammatory cells. Second swab/drop of aspirate was used for culture by inoculating it on routine media like Blood Agar, Nutrient Agar and MacConkey’s agar, incubated at 37° C for 24 hrs. [7-9] aerobically. The plates were examined the next day for growth. Plates not showing any growth were further incubated at 37°C aerobically for another 24 hrs. Plates not showing any growth after 48 hrs on aerobic incubation were considered to be lacking aerobic bacterial pathogens. Smears were made, stained by Gram stain and examined under oil immersion microscope 100X objective.

Antibiotic sensitivity testing was performed on Mueller Hinton Agar according to CLSI guidelines. MRSA was detected using Cefoxitin (30ug) disc and ESBL production in Gram negative bacteria was detected by using Potentiated Disc Diffusion Test (PDT) [8-10]

Exclusion Criteria: Patients already on antibiotics were excluded from the study

Results

One hundred & eight cases of wound infections were taken to isolate and identify the aerobic bacterial pathogens from various departments like Surgery, Gynecology & Orthopedics. Surgical wound swabs were 39 (36.11%) and Non Surgical wound swabs were 69(63.89%) in number.

Out of 69 Non-surgical wounds, 46(66.5%) were soft tissue infections wound and burn wounds, and 23(33.55%) were traumatic wounds. Out of 39 surgical wounds, 21(54.9%) were post-operative wounds and 18 (45.5%) were surgical site infection wounds.

Pus discharge was collected from 108 patients were identified, out of which 67(59.1%) were males and 43(40.9%) were females. Age ranged from 10 months to 65 years with maximum number of cases seen between the age group of 21-40 years (62.3%) as shown in Table 1. Cases of pus discharge came mainly from rural areas (92/85.32%) as compared to urban areas (16/14.68%).

Bacterial Isolates: Out of 108 pus samples, 101(88.5%) were culture positive for bacterial growth and no growth was observed in 7(11.4%) cases as shown in Table 2. Out of 101 bacterial culture positive cases, 96 were monobacterial and 5 were poly bacterial. Out of 96 bacterial isolates; S. aureus (38/36.6%) was the commonest followed by P. aeruginosa (26/24.7%). The prevalence of monomicrobial isolates from various wound infections is depicted in table 3, wherein Staphylococcus aureus was the predominant organism when samples were collected from post operative wounds, burns, traumatic wounds and soft tissue infections.

Antibacterial Susceptibility Profile: Gram positive bacteria showed maximum susceptibility to Vancomycin and Amikacin whereas gram negative isolates showed maximum susceptibility to Imipenem and Amikacin. P. aeruginosa isolates showed maximum susceptibility to Imipenem and Ceftriaxone. 31.4% of S.aureus isolates were MRSA and 36% of Gram negative isolates were ESBL producers as shown in Table 4.

Table 1: Age and Sex Distribution of Wound Infections.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total (no. of cases)</th>
<th>Percentage % (n=108)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>% age</td>
<td>No. of cases</td>
<td>% age</td>
</tr>
<tr>
<td>0-20</td>
<td>11</td>
<td>17.83</td>
<td>06</td>
<td>14.73</td>
</tr>
<tr>
<td>21-40</td>
<td>37</td>
<td>55.41</td>
<td>30</td>
<td>72.63</td>
</tr>
<tr>
<td>41-60</td>
<td>16</td>
<td>22.29</td>
<td>04</td>
<td>10.52</td>
</tr>
<tr>
<td>Above 60</td>
<td>03</td>
<td>4.45</td>
<td>01</td>
<td>2.1</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>100</td>
<td>41</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Distribution Pattern of Bacterial Isolates (n= 108).

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>35</td>
</tr>
</tbody>
</table>
Organisms | Total No. of cases
---|---
Pseudomonas aeruginosa | 23
Escherichia coli | 11
Klebsiella pneumoniae | 07
Proteus mirabilis | 05
Acinetobacter spp. | 04
Coagulase negative Staphylococci | 05
Enterococcus fecalis | 04
Citrobacter freundii | 02
P.aeruginosa + S.aureus | 02
P. aeruginosa + K.pneumoniae | 01
S. aureus + P. mirabilis | 01
K.pneumoniae + P. mirabilis | 01
No growth | 07

Table 3: Monomicrobial isolates in various wound infections.

<table>
<thead>
<tr>
<th>Types of wounds</th>
<th>S.aureus</th>
<th>P.aeruginosa</th>
<th>E.coli</th>
<th>K.pneumoniae</th>
<th>P.mirabilis</th>
<th>Acinetobacter spp.</th>
<th>CONS</th>
<th>E.fecalis</th>
<th>C.freundii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post operative wounds</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Burns</td>
<td>5</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Traumatic</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Soft tissue infections</td>
<td>10</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>23</td>
<td>11</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>36.4%</td>
<td>23.9%</td>
<td>11.4%</td>
<td>7.2%</td>
<td>5.2%</td>
<td>4.1%</td>
<td>5.2%</td>
<td>4.1%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Table 4: Antibiotic Sensitivity Pattern of Bacterial Isolates.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Gram positive isolates n =44</th>
<th>Gram negative isolates n =25</th>
<th>Pseudomonas isolates n=23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin (VA)</td>
<td>100%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clindamycin (CD)</td>
<td>26.5%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linezolid (LZ)</td>
<td>76.5%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>55.9%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin (AMP)</td>
<td>61.8%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amoxyclav (AMC)</td>
<td>52.9%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ceftriaxone (CTR)</td>
<td>73.5%</td>
<td>55.6%</td>
<td>72%</td>
</tr>
<tr>
<td>Cefoxitin (CX)</td>
<td>70.6%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>-</td>
<td>44.4%</td>
<td>-</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>-</td>
<td>38.9%</td>
<td>61.1%</td>
</tr>
</tbody>
</table>
Antibiotics & Gram positive isolates n = 44 & Gram negative isolates n = 25 & Pseudomonas isolates n = 23
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Gentamicin (GEN) & 41.2% & 38.9% & 33.3%
Amikacin (AK) & 82.4% & 72.2% & 69.4%
Imipenem (IPM) & - & 94.4% & 86.1%
Piperacillin + Tazobactum (TZP) & - & 66.7% & 66.7%
Cefpodoxime (CPZ) & - & 50% & -
Ciprofloxacin (CIP) & - & 33.3% & 36.1%
Netlimicin (NET) & - & - & 44.4%
MRSA & 31.4% & - & -
ESBL producers & - & - & 36%

Discussion
In the present study an attempt was made to study the bacteriological profile of wound infections and antimicrobial susceptibility pattern of the isolates. In this study along with the identification of aerobic bacterial organisms, changing pattern of antibiotic sensitivity with special reference to Methicillin Resistant Staphylococcus aureus (MRSA) and Extended Spectrum of Beta lactams (ESBLs) were also Identified.

It was observed that the commonest age group affected is 21-40 years which is correlated with the studies done by Shute Malik et al [11] and Dr. Zarrin Afroz et al [12]. Males (63.4%) were affected more than females (36.6%). This study was correlated with Ramesh Rao et al [13] which showed males (60%) more affected than females, N. Sowmya et al [14] 66.6% and Shruti Malik et al [11] 51.9% also showed the predominance of males over females probably because of their more exposure to life.

Monomicrobial etiology was more common 88.8%, than polymicrobial 4.6%. This study is correlated with N. Sowmya et al [14] 91.7% and Mehta V.J. et al [15] 70.4% wherein the monomicrobial etiology was more common than polymicrobial which may be due to the prior use of antibiotics.

In the present study Staphylococcus aureus 32.4% was the predominant organism followed by Pseudomonas aeruginosa 21.2%, Escherichia coli 10.1%, Klebsiella pneumoniae 6.4%, Proteus mirabilis 4.6% and CONS 4.6%. Staphylococcus aureus (32.4%) was the most predominant isolate which correlated with the other studies done Shruti Malik (30.1%), Mehta V.J. (38.3%), Ramesh Rao (27.8%) and Dr. Pravin (48.4%). [13-16]

Second most predominant organism in the present study was Pseudomonas aeruginosa 21.2% which correlated with the studies of Gayathree Naik (20%), Shruti Malik (17.8%), Mehta V.J (21.3%), Ramesh Rao (18.5%) and Dr. Pravin (17.52%). [11-15]

In the present study, polymicrobial 5 cases included combination of Pseudomonas aeruginosa with Staphylococcus aureus (40%) which correlates with the study of Anbumani et al. Other polymicrobials included Pseudomonas aeruginosa with Klebsiella pneumonia accounting for 20% cases, Klebsiella pneumonia with Proteus mirabilis 20% and Staphylococcus aureus with Proteus mirabilis 20% case.

Out of 35 Staphylococcus aureus isolates, 11(31.42%) were MRSA producers and remaining 24 (68.5%) were MSSA producers. The present study correlates with the study of Rajaduraipandi et al [17] with 31%, Anupurba et al [18] with 32% and N. Soumya et al [14] with 27.5%, as MRSA producers. Among 25 Enterobacteriaceae isolates, 9(36%) were ESBL producers and 16(64%) were Non-ESBL producers which correlates with the studies done by Mehta V.J [15] with 44.6%, K. Rajaduraipandi et al [19] with 40% and Anupurba S et al [20] with 41.2% as ESBL producers respectively.

In the present study Vancomycin (100%) was the most sensitive antibiotic among all gram positive isolates which was correlated with the studies of Amrita Shriyan et al [21], Shahnooshi Javed et al [22] and Jeena Amatya et al [23]. Amikacin was the second most sensitive antibiotic to many gram positive as well as gram negative isolates accounting for 83.5%, which is correlated with the study of Amrita Shriyan et al [21] 95% and Shruti Malik et al [11] 90%. Imipenem was the most sensitive drug among gram negative isolates accounting for 93.1% which was correlating with the study of Shruti Malik et al [11], Amrita Shriyan et al [21] and Ramesh Rao et al [13]. Second most common sensitive drug was Amikacin 79.8% which was
correlated with the study of Shruti Malik et al, Ramesh Rao et al, Jeena Amutya et al and Rai S et al.[11,13,23,24]

**Conclusion**
Wound infections are one of the most common hospital acquired infections, and are an important cause of morbidity & account for 70-80% mortality. Development of such infections represent delayed healing, cause anxiety & discomfort for patient, longer stays at hospitals & add to cost of healthcare services significantly. The incidence of multi drug resistant pathogens as a cause of wound infection is rising. Here lies the importance of formulating an institutional antimicrobial policy based on local microbiological data.

This study was carried out to determine the antibacterial susceptibility of bacteria isolated from wound infections as well as update the clinicians in the various antimicrobial alternatives available in the treatment of wound infections, thus helping to reduce the burden of infection on patients and in long term, it may reduce the cost of treatment. The study concludes that variety of aerobic bacteria is responsible for wound infection with predominance of *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*. Antibiotic sensitivity pattern of the study revealed that Amikacin was the most sensitive drug among both gram positive and gram negative isolates. Imipenem was the most sensitive drug among all gram negative isolates and Vancomycin was the most sensitive drug among gram positive isolates.

More comprehensive studies are required from time to time to define the magnitude of problem & produce data for policy decision on optimal intervention modalities.

**References**
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Financial or other Competing Interests: None.