Wound Infection and Antibiotic sensitivity
Pattern of Bacterial Isolates

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ABSTRACT

BACKGROUND
Wound infection occurs if the integrity and protective function of the skin is breached. Most bacteria, certain viruses (e.g. Herpes virus), Fungi (e.g. Candida albicans) are responsible for wound infection. A retrospective study was designed to isolate and identify the causative agents of wound infection as well as to determine their antibiotic sensitivity pattern.

METHOD
852 pus samples were collected and immediately inoculated on Blood agar and Mac Conkey agar plates. Then the culture plates were incubated at 37°C for 24 hours. After incubation, all isolates were identified by using Gram stain and biochemical methods. Sensitivity tests were performed on Mueller Hinton agar plate by Kirby Bauer’s Disc Diffusion Technique.

RESULT
During the study period (January 2008- July 2009), a total of 852 samples were analyzed. Among 852 samples, 426(50%) showed positive growth. Fifteen different bacterial species were isolated. The most frequent isolate was Staphylococcus aureus (41.31%) followed by Escherichia coli (20.89%), Coagulase Negative Staphylococci (15.72%), Streptococcus viridans (9.62%), Citrobacter species (4.92%), Pseudomonas aeruginosa (3.52%), Enterobacter species (2.11%), Pseudomonas species (1.87%), Proteus mirabilis (0.93%), Proteus vulgaris (0.93%), Streptococcus pyogenes (0.70%), Klebsiella species (0.46%), Salmonella typhi (0.23%) and Streptococcus pneumoniae (0.23%). In invitro sensitivity test of the isolates showed that Cloxacillin (98.53%) and Erythromycin (97.06%) were the most effective antibiotics for Gram positive bacteria and Amoxycillin (65.20%) was the least effective antibiotic. Escherichia coli was the most sensitive to Amikacin (97.06%) followed by Gentamycin (82.02%). All other Gram negative isolates were also the most sensitive to Amikacin (88.88%) followed by Gentamycin (73.85%). Amoxycillin (26.79%) was the least sensitive antibiotic in this study.

CONCLUSION
The most common isolate in wound infection was Staphylococcus aureus followed by Escherichia coli (E.coli), Coagulase Negative Staphylococci (CoNS), Streptococcus viridans, Citrobacter species, Pseudomonas aeruginosa and others. On invitro sensitivity testing, Cloxacillin and the Erythromycin were the most effective antibiotics for Gram positive isolates and Amikacin was the most effective antibiotic for Gram negative bacteria.

Key Words: Wound infection, Staphylococcus aureus, Escherichia coli

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INTRODUCTION:

If the integrity and protective function of the skin is breached, large quantities of different cell types will enter the wound and initiate an inflammatory response. This may be characterized by the classic signs of redness, pain, swelling and fever.\(^1\) The whole process is termed as wound infection.

The potential for infection depends on a number of patients’ variables such as the state of hydration, nutrition and existing medical conditions as well as extrinsic factors such as pre, intra and post operative care if the patient has undergone surgery. Thus it is difficult to predict which wounds will become infected.\(^2\)

Most of the bacteria, certain viruses (e.g. Herpes virus) and fungi (e.g. Candida) are commonly isolated organisms from wound infection. Wound infections remain a major source of postoperative morbidity, accounting for about a quarter of the total number of wound infections. The rate of incidence of Hospital acquired infection related to surgical wounds is as high as 10%.\(^3\)

Wound infections are mainly of two types i.e. open wounds and closed wounds.\(^4\) Open wounds are caused by external damage to intact skin whereas closed wound is infection to tissues below the skin.\(^5\), \(^6\).

Human skin acts as an excellent barrier to infection, provided it is not breached. However certain parasites (hook worm larvae) and most other bacteria can penetrate the skin.\(^7\) Some primary skin infections like Impetigo are caused by Streptococcus pyogenes or Staphylococcus aureus.\(^8\) Boils and furuncles are the commonest suppurative lesions with necrosis in the centre. Carbuncle is a large abscess usually occurs in thick collagenous tissue such as back of the neck. The boils, furuncles and carbuncles are caused by Staphylococcus aureus. Cellulitis is a relatively deep infection involving subcutaneous spaces to dermis caused by group A streptococci, Staphylococcus aureus(S. aureus) and Haemophilus influenzae.\(^9\) Certain viruses (Human papilloma virus), fungi (Trichophyton species) and yeast (Candida albicans) are also responsible for primary infection. Other Gram negative bacteria including Pseudomonas aeruginosa (Paeruginosa), E.coli, and Klebsiella species are responsible for secondary wound infection.\(^10\), \(^11\).

Infections of the surgical wound are one of the most common hospital acquired infections and are important cause of morbidity and mortality. The delay in recovery and subsequent increased length of hospital stay also has economic consequences.\(^12\) During hospitalization the patients skin flora changes which increases the risk of infections from hospital strains of common pathogens mainly during post operative condition.\(^3\).

METHODOLOGY

This is a retrospective study of pus samples from all wounds over a period of 19 months from January 2008 to July 2009. Total 852 samples were collected from patients visiting Kathmandu Model Hospital. Pus samples were collected with the help of sterile disposable cotton swabs and immediately inoculated onto Blood agar and McConkey agar media and incubated at 37°C for 24 hours. After incubation, identification of bacteria from positive cultures was done with standard microbiological technique which included Gram staining and biochemical reactions. The antibiotic sensitivity test of all isolates was performed by modified Kirby Bauer’s disc diffusion method on Mueller Hinton agar or Blood agar medium using antibiotic discs of Hi media Laboratories Pvt Limited, India and Oxoid Diagnostics, UK.

RESULT

Out of total 852 samples collected, 531 (62.32%) samples were from male patients and 321 (37.67%) samples from female patients. Among 531 samples from male, 248 (58.21%) samples showed positive growth and among 321 samples from female 178 (51.78%) showed positive growth.

Out of 852 samples tested, 426 (50%) samples showed bacterial pathogens responsible for wound infection. Among the total isolates 273 (64.08%) were Gram positive bacteria and 153 (35.92%) were Gram negative bacteria.

Out of 426 isolates, Gram positive bacteria were predominant accounting 273 (63.81%) of the total isolates. Among the Gram positive isolates, S. aureus was the most frequently isolated species with 176 (60.48%) and 4 (1.37%) isolates were found to be Meticillin Resistant Staphylococcus aureus (MRSA). Similarly, Gram negative organisms constituted 153 (33.55%) of total isolates. Among the Gram negative
isolates, *E. coli* (58.16%) was the most frequent isolate. The data was followed by *Citrobacter species, Pseudomonas aeruginosa* and *Enterobacter species*.

Among the Gram positive bacteria, the most sensitive antibiotics were Cloxacillin (90.90%) and Erythromycin (90.34%) followed by Cefotaxime (88.06%) and Ciprofloxacin (84.65%). The least effective antibiotic was Amoxycillin (51.13%).

Among 153 Gram negative isolates, the most effective antibiotic was Amikacin (88.88%) followed by Gentamycin (73.85%), Ciprofloxacin (72.54%) and the least effective antibiotic was Amoxycillin (26.79%).

**DISCUSSIONS:**

Any wound is at some risk of becoming infected. When a wound fails to heal, the patient suffers increased morbidity, treatment costs rise and general wound management practices become more resource demanding. As wound infection is becoming the major hospital acquired infection, hospital environment plays a major role for causing wound infection.

In our present study, 50.00 % of pus samples showed bacterial growth with 15 different bacterial species and the most common isolate was *Staphylococcus aureus* (41.31%) followed by *E. coli* (20.89%), CoNS (15.44%)
and Streptococcus viridans (9.62%). In a similar study conducted in TUTH found that 82.50% showed the bacterial growth and 13 different bacterial species were isolated including predominantly *Staphylococcus aureus* (57.70%) followed by *E. coli* (11.00%) and CoNS (3.00%)12. Lesser percentage of growth positive in our study may be due to delay in transportation of samples to laboratory from the site of collection. The another most probable reason is the collection of samples from patients taking antibiotics. Similar study was also conducted in Medicare National Hospital and Research Centre where 60% was found to be growth positive with the isolation of 7 different bacterial species. Most common isolate was again *Staphylococcus aureus* (50%) followed by *E. coli* (11.7%), *Pseudomonas aeruginosa* (8.3%), *Streptococcus pyogenes* (8.3%), *Klebsiella pneumoniae* (6.7%), CoNS(6.7%) and Proteus species (5%)13. Another study carried out at TUTH from 15th June to 15th December 2004 to determine the drug sensitivity pattern of microorganisms from infected wounds. In this study also the common isolate was *Staphylococcus aureus* (57.25%) followed by *E. coli* (15.61%)14. In another study carried out at Tertiary Care Hospital in Turkey from January 1999 to January 2004 to determine the spectrum of the pathogens from surgical wound infections. Out of 621 pathogens isolated, the most common isolate was *Staphylococcus aureus* (50%)15.

In vitro sensitivity testing of our study showed that Cloxacillin (96.02%) was the most effective antibiotic against *Staphylococcus aureus* followed by Erythromycin (90.90%) and Cefotaxime (88.06%) and the least effective antibiotic was Amoxycillin (51.13%). Among the Gram negative bacteria, *E. coli* was the most sensitive to Amikacin (88.88%) followed by Gentamycin (73.85%) and Ciprofloxacin (72.54%). The least effective antibiotic was Amoxycillin (26.79%) and Ceftriaxone (39.21%). Our data is supported by the study conducted at TUTH and Medicare National Hospital and Research Centre13,14. In our study *Citrobacter species* was the most sensitive to Amikacin (95.23%) followed by Ciprofloxacin (95.23%) and Cotrimoxazole (76.19%). The least effective antibiotic for *Citrobacter species* was Amoxycillin (14.28%) and Ceftriaxone (19.04%). For *Pseudomonas aeruginosa*, the most effective antibiotic was Amikacin (93.33%) and Gentamycin (86.66%). The least effective antibiotic was Ceftriaxone (26.66%) followed by Cefotaxime (33.33%). Our results are reinforced by the study conducted in United States where more than 90% isolates were Susceptible to Amikacin, 70-80% were susceptible to Gentamycin and Ciprofloxacin16.

**CONCLUSION:**

The most common isolate in wound infection was *Staphylococcus aureus* followed by *E. coli*, CoNS, *Streptococcus viridans, Citrobacter species, Pseudomonas aeruginosa* and others. On invitro sensitivity testing, Cloxacillin and the Erythromycin were the most effective antibiotics for Gram positive isolates and Amikacin was the most effective antibiotic for Gram negative bacteria. Indiscriminate use of antibiotic has lead to the development of antibiotic resistant strains for commonly used antibiotics such as Quinolones and Cephalosporins. Thus; a detail study is required with regard to proper antibiotic usage, susceptibility testing irrespective of the organisms isolated from pus to find out the resistance pattern.

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**Table 2: Antibiotic Sensitivity Test of Gram Positive Isolates**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S. aureus (N=176)</th>
<th>CoNS (N=67)</th>
<th>S. viridans (N=41)</th>
<th>S.pyogens (N=3)</th>
<th>MRSA (N=4)</th>
<th>S.pneumoniae (N=1)</th>
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<tbody>
<tr>
<td>Amoxycillin</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>90</td>
<td>86</td>
<td>51</td>
<td>16</td>
<td>30</td>
<td>11</td>
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<td>Ciprofloxacin</td>
<td>149</td>
<td>27</td>
<td>58</td>
<td>9</td>
<td>38</td>
<td>3</td>
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<tr>
<td>Cotrimoxazole</td>
<td>145</td>
<td>29</td>
<td>60</td>
<td>7</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>Cephalexin</td>
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<td>30</td>
<td>52</td>
<td>15</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>Cloxacillin</td>
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<td>17</td>
<td>60</td>
<td>7</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>160</td>
<td>16</td>
<td>60</td>
<td>7</td>
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<td>3</td>
</tr>
</tbody>
</table>

S = Sensitive   R = Resistant

**Table 3: Antibiotic Sensitivity Test of Gram Negative Isolates**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E.coli (N=89)</th>
<th>Citrobacter sps (N=21)</th>
<th>P. aeruginosa (N=15)</th>
<th>Enterobacter sps (N=9)</th>
<th>Pseudomonas sps (N=8)</th>
<th>P.mirabilis (N=4)</th>
<th>P.vulgaris (N=4)</th>
<th>Klebsiella sps (N=2)</th>
<th>S.typhi (N=1)</th>
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<tr>
<td>Amikacin</td>
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<td>5</td>
<td>20</td>
<td>1</td>
<td>14</td>
<td>1</td>
<td>7</td>
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<tr>
<td>Amoxycillin</td>
<td>31</td>
<td>58</td>
<td>3</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>-</td>
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<td>63</td>
<td>33</td>
<td>16</td>
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<td>10</td>
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<td>10</td>
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<td>4</td>
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<td>8</td>
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<tr>
<td>Cotrimoxazole</td>
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<td>8</td>
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<td>Ceftriaxone</td>
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<td>55</td>
<td>12</td>
<td>4</td>
<td>17</td>
<td>11</td>
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<td>5</td>
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</tbody>
</table>

S = Sensitive   R = Resistant

**REFERENCE:**