Antibiotic Sensitivity profile of bacteria isolated from wounds and skin infections

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Background: The choice of antibiotic is usually based on previously published susceptibility testing and previous clinical success. Anaerobic bacteria are important because they dominate the diagnose flora. Some of these infections are serious and have high mortality rate. It has to be paid more attention to anaerobic infections because special precautions are needed for appropriate collection and transport of specimens. Aim of the study: To study the sensitivity profile of bacterial strains isolated from several types of infected skin and wounds toward several antibiotics. Methods: The study included 98 strains isolated from 150 patients with skin and wound infections patients hospitalized into Al-Diwanya teaching hospital from January 2009 to January 2010. A total of 150 samples were selected from patients with skin and wounds infections including samples of burn wounds, cutaneous ulcer, surgical wounds, acnes and pustules. For the disc diffusion testing: ampicillin, gentamicin, amikacin, cefuroxime, cefoperazone, ciprofloxacin, pipercillin, Erythromycin, Cloxacillin, ceftriaxone, ceftazidime, Amoxicillin, Rifampin, Metronidazole, clindamycin, Cefazidim, penicillin V, trimethoprim-sulfamethoxazol and amoxicillin _ clavulanic acid, disc were used.

Results: In our research, we examined 150 specimens. Aerobic bacteria were recovered from 48 (32%) specimens, anaerobic bacteria only were recovered from 27 (18%) specimens, and mixed aerobic and anaerobic bacteria were recovered from 75 (50%) specimens. Ciprofloxacin was highly active drug that could eradicate the major pathogens bacteria from skin and wounds infection. Although metronidazole has relatively poor efficacy by itself, in combination with penicillin V the susceptibility of the bacteria in this study was virtually the same as amoxicillin.

Conclusions: Ciprofloxacin seems to remain the antibiotic of choice because of its efficacy in polymicrobial infections, relatively narrow spectrum for bacteria found in skin and wounds infections, low toxicity, and low cost.

Introduction

Antibiotics are often prescribed for the adjunctive treatment of skin and wounds infections. The choice of antibiotic is usually based on previously published susceptibility testing and previous clinical success. There is concern that bacteria have increased resistance to the currently prescribed antibiotics (1).

Susceptibility testing is the determination of the bacterial pattern of resistance to a number of antibiotics. It would be ideal if susceptibility testing could always be undertaken before the prescription of antibiotics. Unfortunately, it usually takes from several days to weeks to cultivate and do susceptibility tests on anaerobic bacteria (2).

Anaerobic bacteria are important because they dominate the diagnosed flora. They are commonly found in different infections. Some of these infections are serious and have high mortality rate (3, 4 and 5). It has to be paid more attention to anaerobic infections because special precautions are needed for appropriate collection and transport of specimens. Isolation and identification of anaerobic bacteria can be complex, difficult, labor intensive, and expensive. The majority of these infections have caused mixtures of numerous strains of aerobic and
anaerobic bacteria. Interpreting culture to establish the extent, to which any one particular anaerobe in the mixture is contributing to infection, is difficult (6 and 7). Treatment considerations for these mixed anaerobic infections are difficult and causing even more problem with increasing resistance among these groups of organisms. A number of antimicrobials have poor or no activity against some bacteria (8, 9 and 10). Failure to provide antibacterial coverage against the anaerobes in a mixed aerobic-anaerobic infection may lead to inadequate response. This could, of course, be attributed to another factor such as the possibility of an untrained abscess (11). The aim of the present study was to study the sensitivity profile of bacterial strains isolated from several types of infected skin and wounds toward several antibiotics.

Methods:

The study included 98 strains isolated from 150 patients with skin and wound infections patients hospitalized into Al-Diwanya teaching hospital from January 2009 to January 2010. A total of 150 samples were selected from patients with skin and wounds. Infections including samples of burn wounds, cutaneous ulcer, surgical wounds, acnes and pustule were examined. For the disc diffusion testing (2): ampicillin, gentamicin, amikacin, cefuroxime, cefoperazone, ciprofloxacin, piperacillin, Erythromycin, Cloxacillin, ceftriaxone, ceftazidime, Amoxicillin, Rifampin, Metronidazole, clindamycin, Ceftazidim, penicillinV, trimethoprim-sulfamethoxazol and amoxicillin _ clavulanic acid, disc were used.

Collecting of specimens was done with syringe and swabs. All of specimens were transferred to transport media. Swab specimens were homogenized in a small amount of broth. Aspirates were thoroughly mixed before inoculation. For transport media Tripticasoy broth for aerobic bacteria and Thioglycolate broth for anaerobic bacteria were used. Purulent secretions were cultivated on Nutrient Broth (37°C, 24 h), and then transferred on selective media (blood-agar, MacConkey agar, Levin Eosin Methylene Blue Agar(Levin EMB Agar) , Cystine Lactose Electrolyte Deficient Agar(CLED-agar) and subsequently on average multitest media (MIU, TSI, Simmons). For the final identification we used API 20E Biomerieux tests (12 and 13).

Sheep blood (5%), chocolate, and MacConkey agar plates were inoculated for the isolation of aerobic organisms. The plates were incubated at 37°C aerobically (MacConkey agars) or under 5% carbon dioxides (blood and chocolate agar) and examined at 24 and 48 h. For the isolation of anaerobes, specimens were plated onto prerereduced, vitamin K1-enriched brucella blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin; or an anaerobic blood plate containing colistin and nalidixic acid and then inoculated into enriched thioglycolate broth (14).

The plated media were incubated in anaerobic incubator condition and examined after 48, 72, 96, and 120 h. After growing of the colonies; we stained colonies of bacteria with gram staining and determined shape of bacteria. Then we used specific culture and test for identifying type of bacteria. The thioglycolate broth was incubated for 14 days. Aerobes and anaerobic bacteria were identified by techniques previously described, (15).

In the mean time we used aerobic and anaerobic condition. When we identified type of bacteria which caused infections, we performed antibiogram test by Kirby-Bauer method (gel diffusion test) in blood or chocolate agar with Muller-Hinton base agar. After 24 h for aerobic and 48-72 h for anaerobic bacteria, we reported susceptibility of bacteria to antibiotic disk.

Results:

One-hundred fifty samples from patients with burn wounds 56(37.3%), cutaneous ulcer 40(26.7%), surgical wounds 31(20.7%), acnes 14 (9.3%) and 9(6%) pustules were examined (Figure 1). Specimens were 88 from females and 62 from males with age between 10-60 years old (Figure 2).
Ninety eight isolates were recovered: 65(66.3%) aerobes and 33(33.7%) anaerobes (Table 1). A total of 65 aerobic isolates were recovered. The predominant ones were *Staphylococcus aureus* (n12), group A Streptococci (n 6), *Escherichia coli* (n17), *P.aeruginosa* (n7), *Proteus mirabilis* (n6), *Proteus vulgaris* (n4), Non-coagulase *S. aureus* (n2), *Enterococcus* sp. (n 4), *Acinetobacter* sp. (n 3), *Enterobacter* sp. (n 2), and *Citrobacter* sp. (n2). A total of 33 anaerobic
bacteria were recovered *Peptostreptococcus* sp.(n10), *Propionibacterium acnes* (n9), *Fusobacterium* (n5), *Clostridium* sp. (n5), and *Bacteroides* sp. (n 4) (Table 1).

**Table 1. Bacteria isolate from patients with skin and wounds infections.**

<table>
<thead>
<tr>
<th>Aerobic and facultative bacteria</th>
<th>No. of isolates</th>
<th>Anaerobic bacteria</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus spp</em></td>
<td>4</td>
<td><em>Peptostreptococcus spp</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12</td>
<td><em>Propionibacterium acnes</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>6</td>
<td><em>Fusobacterium</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>17</td>
<td><em>Clostridium spp</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>6</td>
<td><em>Bacteroides spp</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Citrobacte spp</em></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-coagulase S. aureus</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter spp.</em></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>Total</td>
<td>33</td>
</tr>
</tbody>
</table>

Susceptibility of these aerobic bacteria was as following: the sensitivity of Ampicillin 93%, cefoperazone 90%, ciprofloxacin 100%, amikacin 60%, cefuroxime68%, piperacillin 80%, Erythromycin 92%, and ceftazidime 90%. And their resistance to Cloxacillin 75%, ceftriaxone 90% and Gentamycin was 88% (Figure 4).
Figure 4. The antibiotic susceptibility of aerobic bacteria isolated from patients with skin and wounds infections.

In anaerobic bacteria, sensitivity to Ciprofloxacin was 100%, clindamycin 92%, Ceftazidim 80%, Amoxicillin 90%, Rifampin 80%, penicillin V 70%, and Metronidazole 45%. And their resistance to Erythromycin was 78%, ceftriaxone 88%, Cloxacillin 91% and Gentamycin 87% (Figure 5).

Figure 5. The antibiotic susceptibility of anaerobic bacteria isolated from patients with skin and wounds infections.
In combination antibiotic therapy used to treat the anaerobic bacteria isolates, the percentage of susceptible for the combination of amoxicillin+ clavulanic acid was 100%, amoxicillin+ metronidazole 98% and penicillin V+ metronidazole 95%, and trimethoprim-sulfamethoxazol 92% (Figure 6).

Sensitivity of both anaerobic and aerobic bacteria to Ciprofloxacin was 100%, so we suggest this drug for treatment of many infections.

**Figure 6.** The combination of antibiotic susceptibility of anaerobic bacteria isolated from patients with skin and wounds infections.

**Discussion**

The prescription of antibiotics should be adjunctive to appropriate clinical treatment. Antibiotics are indicated when signs and symptoms are associated with systemic involvement, for patients with progressive infections, or for patients who are immunocompromised (16). Selection of an antibiotic regimen should be based on knowledge of the efficacy of an antibiotic for the bacteria most often associated with severe infections. Thus, if an antibiotic is effective against some species of bacteria (17). Thus, if an antibiotic is effective against some species of bacteria in a polymicrobial infection, it may indirectly affect other bacteria in that ecosystem.

In many studies of skin and soft tissue, *Staphylococcus aureus* was the most common pathogen. Group A *Streptococci* ranks as a second common pathogen in gram positive cocci (18, 19 and 20). In our study we found *S. aureus* (18.5%) from all aerobic bacteria and *Streptococcus pyogenes* (9.3%). Other reports found *S. aureus* (37.3%) and streptococcus pyogenes (16.3%) (21). Other reports showed that the isolation rates of *Bacteroides Fragilis* group organism have recently been increasing in both primary and post-operative infection (18, 22 and 19) and *Peptostreptococci* typically are the most common isolated anaerobic bacteria (23 and 24). We isolated *Peptostreptococci* (30.3%) and *Propionibacterium acnes* (27.3%), which is as same as the other reports.

Nevertheless, accurate information regarding the efficacy of a certain agent in inhibiting or killing the organism will certainly give useful clinical information for choice of a therapeutic
A consensus group of infectious disease clinicians concluded that in the most serious infections involving anaerobes, susceptibility test results correlate with the clinical response.

The mechanisms by which anaerobic bacteria become resistant to lactamase antibiotics are similar to those described in aerobes and include the production of B-lactamases, changes in penicillin G binding proteins, and changes in outer membrane permeability to lactamases (25 and 22). Antibacterial therapy must cover the key pathogens. Some compounds have significant activity against both aerobic and anaerobic microorganisms (18, 19, and 20). In our study, it was shown that aerobic bacteria resistance rate were: ceftriaxone (90%) Gentamycin (88%), Cloxacillin (75%). And sensitivity were Ciprofloxacin (100%), Ampicillin (93%), cefoperazone (90%), Erythromycin (92%), and ceftazidime (90%).

In anaerobic bacteria, resistance rate were: Ertromycin (78%), ceftriaxone 88%, Cloxacillin 91% and Gentamycin 87%. And sensitivity was: Ciprofloxacin 100%, clindamycin 92%, Ceftazidim 80%, Amoxicillin 90%, Rifampin 80%, penicillin V 70%, and Metronidazole 45%. Clindamycin had efficacy against (92%) of anaerobic bacteria in this study. Other studies on this topic report a sensitivity of 96% to Clindamycin (26).

Previous studies have also demonstrated the efficacy of clindamycin, and it is often recommended for the treatment of serious surgical and wounds infections when penicillin is contraindicated or in cases in which penicillin therapy has failed (16 and 27).

Metronidazole is a nitroimidazole compound, which was developed to treat protozoan infections but later was found to be effective against anaerobes. Metronidazole has been recommended for use in combined therapy with penicillin to treat surgical and wounds infections (16). Of the antibiotics tested in this study, metronidazole had the greatest amount of bacterial resistance (55%). Other studies report the same resistance (55%) to metronidazole (26).

Metronidazole is only active against anaerobes and should not be used alone for the treatment of skin and wounds infections, which also contain facultative bacteria. Metronidazole is only active when it is reduced to form an unstable intermediate that binds to microbial DNA and produces damage that prevents replication and transcription. If metronidazole were used in combination with penicillin V, the percentage of susceptible bacteria would increase from 45% to 95%. If metronidazole were used in combination with amoxicillin, the percentage of susceptible bacteria would increase from 45% to 98%. Thus, the combination of amoxicillin and metronidazole continues to have clinical efficacy even with the relatively high incidence of resistance to metronidazole. In this study, (10%) of anaerobic bacteria were resistant to amoxicillin, whereas (20%) were resistant to both Ceftazidim and Rifampin, and none of the anaerobic bacteria were resistant to amoxicillin/clavulanic acid.

**Conclusions**

We concluded that, in skin and wounds infections which are composed of both aerobic and anaerobic bacteria, Ciprofloxacin, was highly active drug that could eradicate the major pathogens bacteria found from skin and wounds infection in Iraqi patients. Because some anaerobes are resistant to penicillin, treatment should also include appropriate coverage of those organisms. In conclusion, the results of the present investigation show a high level of resistance in aerobes and anaerobes bacteria. This may be the result of the extensive antibiotic used in patients.
In conclusion, Ciprofloxacin seems to remain the antibiotic of choice because of its efficacy in polymicrobial infections, relatively narrow spectrum for bacteria found in skin and wounds infections, low toxicity, and low cost.

Although metronidazole has relatively poor efficacy by itself, in combination with penicillin V the susceptibility of the bacteria in this study was virtually the same as amoxicillin. Amoxicillin and amoxicillin/clavulanate did have greater activity for the bacteria isolated in this study than penicillin V by itself. However, amoxicillin and amoxicillin/clavulanate have a wider spectrum of activity than penicillin V.

Clindamycin remains an excellent alternative for patients allergic to the penicillin.

References


