Study of Betalactamase Production in Coagulate Positive Staphylococci by Iodometric and Acidometric Methods and Their Antibiotic Resistance Pattern

M. Bokaeian and M.I. Qureshi

Due to importance of coagulase positive staphylococci in pyogenic infections and their increasing rate of resistance to betalactam antibiotics and also important role of betalactamase enzyme in this phenomenon, we accomplished this study for isolation, measurement of betalactamase enzyme and antibiotic resistance assay by standard methods. Total of 603 different samples obtained from patients with staphyloccocal infections were cultured on general and selective media and after 24-48 h incubation at 37°C, coagulate reaction and biochemical tests were used to identification. Betalactamase assays accomplished by acidometric and iodometric methods and antibiotic susceptibility of isolated strains were done by standard agar diffusion of Kirby Bauer method. Results revealed out of 603 samples, 38 coagulase positive staphylococci were isolated (6.3%). Betalactamase assay by acidometric and iodometric methods were positive in 78.9 and 73.6%, respectively. All of the bacteria were resistant to carbenicillin, ampicillin and amoxyccillin and on other hand resistance of other betalactams include as: penicillin (97.3%), oxacillin and methicillin (18.4%), cefitoxime (17%), cephalothin (15.7%), ceftriaxon (14%), cephalxin (13.1%), cefazolin (7.8%). In accordance with high resistance of isolated coagulase positive staphylococci to carbenicillin, penicillin, ampicillin and amoxyccillin and high rate of betalactamase production in these pathogen bacteria that lead to resistance to betalactam antibiotics we suggest the attention of physicians to the role of the bacteria and their proper treatment in the city.

Key words: Coagulate positive staphylococci, betalactamase, resistance
INTRODUCTION

Coagulase positive staphylococci include *S. aureus* are important pathogens related to family Micrococaceae which responsible for more than 80% of pyogenic infections and are the second cause of nosocomial infections (Hindler and Jorgensen, 2000). Infection by coagulase positive staphylococci can prove difficult to treat either because they have an unpredictable sensitivity pattern or because they are resistant to many beta-lactam antibiotics.

In spite of many antibiotics discovered against them, these bacteria are still of the most important pathogens responsible for problematics in medicine. Beta-lactam antibiotics are of the most important antibiotics which are used for treatment of staphylococcal infections nowadays but unfortunately many of coagulase positive staphylococci are resistant to betalactams in different ways (e.g., betalactamase production, intrinsic resistance and resistance due to tolerance) (Waldvogel, 2000).

Of the all above resistance mechanisms, resistance due to betalactamase production is very important and in accordance with estimates about 70-80% of coagulase positive staphylococci seen outside of hospitals and more than 90% of the hospital strains produce this enzyme (Waldvogel, 2000).

Different methods are used for the detection of beta-lactamases namely iodometric, acidimetric, chromogenic cephalosporin and microbiological method (Sykes, 1979). The iodometric assay is based on the fact that the intact (active) penicillin molecule does not bind iodine whereas the betalactamase inactivated product penicilloic acid binds iodine. Thus a positive reaction indicates that iodine being bound to penicilloic acid is unavailable for further reaction with starch and so no purple colour develops in testing (Lee and Komarny, 1981). The acidimetric method uses citrate-buffered penicillin and phenol red as a pH indicator. When colonies of beta-lactamase positive organism are added to the solution, the penicilloic acid present results in a drop in pH, causing a color change from red to yellow (Hindler and Jorgensen, 2000).

Many commercial companies produce betalactamase measurement kits which provides different methods and their results are almost same and comparable (Gradus and Silver, 1989) although few studies show different results (Fung and Jang, 1995). Because such kits are not used in our country and betalactamase tests are not done in routine labs, so there are no sufficient information about betalactamase production by coagulase positive staphylococci in Zahedan region. This study was done to measure betalactamase enzyme in these bacteria and to determine susceptibility of the bacteria to betalactam antibiotics.

MATERIALS AND METHODS

In this experimental-diagnostic study, a total of 603 different samples collected from patients of central hospital in Zahedan. All patients were examined by specialist. The samples in our study were as 356 blood, 78 cerebrospinal fluid, 56 sputum, 25 wound exudate, 25 abscess, 22 ear discharge, 14 peritoneal fluid, 10 joint fluid, 10 eye discharge and 6 pleural fluid.

After gram staining, all samples were cultured on blood agar and manitol salt agar. After 48 h incubation at 37°C in aerobic atmosphere, suspected colonies were examd by gram staining and coagulase test (Humphreys, 2002). Betalactamase production in confirmed coagulase positive staphylococci were determined by iodometric (tube test) and acidimetric methods (Sonnemirth and Jarret, 1980) and their antibiotic susceptibility patterns were determined by standard Kirby-Bauer disk diffusion method (Humphreys, 2002).

Antibiotics like Oxacillin (1 µg), Carbenicillin (100 µg), Cephalexin (30 µg), Penicillin (10 U), Cephalothin (30 µg), Ceftriaxone (30 µg), Cefizoxime (30 µg), Cefazolin (30 µg), Methicillin (1 µg) and Amoxicillin (25 µg) were used.

RESULTS

Thirty eight coagulase positive staphylococci were isolated (6.3%) from 603 studied samples which include wound exudate (9), sputum (7), abscess (6), ear discharge (5), joint fluid (4), blood (4), eye discharge (2) and cerebrospinal fluid (1) (Table 1).

Betalactamase production test in acidimetric and iodometric methods were positive in 78.9 and 73.6%, respectively (Table 2).

<table>
<thead>
<tr>
<th>Culture specimen</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>352</td>
<td>4</td>
<td>356</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>77</td>
<td>1</td>
<td>78</td>
</tr>
<tr>
<td>Sputum</td>
<td>49</td>
<td>7</td>
<td>56</td>
</tr>
<tr>
<td>Wound exudate</td>
<td>17</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>Abscess</td>
<td>19</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Ear discharge</td>
<td>17</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Joint fluid</td>
<td>6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Eye discharge</td>
<td>8</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>565</td>
<td>38</td>
<td>603</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result test</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidimetry</td>
<td>78.9% (n:38)</td>
<td>21.1% (n:10)</td>
<td>100% (n:38)</td>
</tr>
<tr>
<td>Iodimetry</td>
<td>73.6% (n:28)</td>
<td>26.4% (n:10)</td>
<td>100% (n:38)</td>
</tr>
</tbody>
</table>
Acidimetry detected maximum betalactamase producers in all samples and weak betalactamase producers were detected mainly by acidimetry method. Only 7% of betalactamase producers could be detected by one of two methods used whereas 88% positivity was obtained by using both two methods. This shows that a combination of methods is always useful in detecting betalactamase producers.

All of the bacteria were resistant to carbenicillin, ampicillin and amoxycillin but on other hand resistance of other betalactams include as: penicillin (97.3%), oxacillin and methicillin (18.4%), ceftriaxone (17%), cephalothin (15.7%), ceftriaxon (14%), cephalaxin (13.1%) and cefazolin (7.8%).

DISCUSSION

Positive cultures, most abundance related to wounds, sputum and less prevalence related to cerebrospinal fluid and eye discharge. In regard to coagulase positive staphylococci, different statistical data observed (Saleao, 1999; Hsueh, 1999). There are different idea about this differentiation which include infection in other parts of body and its transmission to sampling sites, variable rate of carriers, resistance to antimicrobial agents and ability to produce disease in hospital environment (Humphreys, 2002).

In present study, significant difference not seen between the two betalactamase measurement methods (p<0.05) which is consistent with the results of other studies that have demonstrated similar results by different methods of betalactamase assay (Gradus and Silver, 1989) but in other study, acidimetry have been reported more reliable than iodometry (Nahae et al., 1999).

The time and expense in acidimetry were less than iodometry in our study and other studies notify that acidimetry is a one-step procedure (Sonnenwirth and Jirett, 1988), nowadays there also another methods of betalactamase assay (Adyem et al., 2000; Troillet et al., 1998; Chen et al., 1994; Ronco et al., 1989).

In present study, 84% of the all coagulase positive staphylococci produced betalactamase determined by at least one of the betalactamase assay methods. Different studies show that at least 70% of coagulase positive staphylococci produce the enzyme (Waldvogel, 2000). On the other hand, almost all of coagulase positive staphylococci in our study shows resistance to carbenicillin, ampicillin, amoxycillin and penicillin. Because such antibiotics are susceptible to betalactamase and because of high rate of betalactamase production, these results would be anticipate. Some of the isolated staphylococci were resistant to these antibiotics while their betalactamase test were negative probably due to other mechanisms of resistance (Waldvogel, 2000; Nahae et al., 1999).

18.4% of isolated staphylococci showed resistance to oxacillin and methicillin. Nowadays meticillin resistant Staphylococcus aureus (MRSA) have been emerged as problematic in medicine which its prevalence differs from region to region (Hsueh, 1999; Saleao, 1999; Troillet et al., 1998).

The least resistance were observed in cephalothin, ceftriaxone, cephalaxin and cefazolin which is probably because of their resistance to betalactamase.

Due to high percentage of resistance of coagulase positive staphylococci to antibiotics such as carbenicillin, ampicillin, amoxycillin and penicillin that correlates with betalactamase production, we suggest use of betalactamase resistant antibiotics such as oxacillin, cloxacillin, cephalothin etc. We also recommend use of betalactamase test (specially acidimetry method) before antibiotic susceptibility testing which could save the time and expense and also gains rapid and important information about use of betalactam antibiotics.

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REFERENCES


