Survey of methicillin-resistant Strains of *Staphylococci* from Neonatal Septicemia for *mecA* gene

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Abstract: septicemia is still prevalent among neonates and it is a major medical problem. The aim of this study was survey of methicillin-resistant strains of *Staphylococci* from blood culture in neonate for *mecA* gene distribution. 138 blood cultures samples performed from neonates and identified the *Staphylococci spp*. These isolates were tested for antimicrobial susceptibility according to CLSI. Detection of *mecA* gene was performed by PCR. Among 138 samples from neonates 31.8% were positive blood culture for *Staphylococci* strains; of which 54.5% and 45.5% were *Coagulase negative Staphylococcus* (CONS) and *Staphylococcus aureus* respectively. total 24 samples of CONS were biotyped, *S. epidermidis* (62.5%) and *S. saprophyticus* (37.5%). maximum resistance was seen with Ampicillin and minimum Resistance with Ciprofloxacin. prevalence of MRCONS was 55.6% and MRSA was 55%.The *mecA* gene was detected in 87% of the isolated CONS and 70% of *S. aureus* isolates. This study show that the high prevalence of methicillin resistance among staphylococci strains in this area of Iran and CONS predominated as the cause of methicillin resistance.

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Key words: Blood culture, Neonates, CONS, mec A, MRSA

Introduction

Septicemia, is a common condition in neonates with a resultant high mortality and morbidity rate in spite of new advances in antibiotic therapy (Yalaz, 2006). *Staphylococcus* are the most abundant isolated bacteria from blood. Since septicemia with *Staphylococcus aureus* is associated with a high mortality and an increased length of stay in hospital, timely detection and identification of *S. aureus* or coagulase-negative *staphylococci* (CONS) including methicillin resistance from the patient’s blood has great therapeutic, economic and prognostic significance (Gröbner and Kempf, 2007). These strains carry the *mecA* gene, which encodes a modified penicillin-binding protein (PBP2a) that is responsible for resistance to β-lactam antibiotics. (Livermore, 2000) Identification of methicillin resistance is performed by phenotypic and genotypic methods (Martins, 2007). Today, the phenotypic method such as disk diffusion is used in most laboratories that various factors affect on the growth of bacteria and results(Mirsalehian A, 2003). Hence a sensitive and exact method is necessary that be independent from conditions of culture media. Isolation mecA gene is a useful marker for identification of resistance to oxacillin in *Staphylococcus spp*.PCR is a rapid and accurate method for isolation this gene.(De Giusti, 1999) Hence, the present study was undertaken to survey of methicillin-resistant blood culture strains of *Staphylococci* from neonates for *mecA* gene distribution in Beasat hospital, Sanandaj, Iran.

Materials and Methods

Total of 138 blood samples were taken from neonates with symptoms suggestive of neonatal sepsis from the newborn ward were included in this study. The antimicrobial susceptibility testing for all the *Staphylococci* strains to various antimicrobial agents were determined according to the Clinical Laboratory Standards Institute.(A, 2007)

**Oxacillin disc Diffusion test**

To determine the antibiotic methicillin resistant strains of *Staphylococcus aureus*, strains on Mueller-Hinton agar medium 4% salt were cultured. Resistance to methicillin by disk diffusion method using oxacillin disk company mast Examined. The pattern of antibiotic resistant strains *MRSA* according to CLSI isolated.(A, 2007)

**PCR application of the mecA gene**

Detection of *mecA* gene was performed by PCR (Shubhra S., 2009). Genomic DNA was extracted by a commercial extraction kit, Sina Gene Company, Tehran, Iran using the Eppendorf Master cycler. Both forward and reverse primer pair was used. The forward primer is GGAATGCAGAAAGACCAAAG while the reverse primer is CTTTGGTCTTTCTGACATTCTCG. Amplification was done using a thermal regime of 35 cycles of amplification at 95°C for 5 min, and 95°C for 45s which was for denaturation. Annealing temperature
was set at 58 °C for 45s. The extension phase was done using a temperature of 72 °C for 1 min and the 2nd one was 72 °C for 10 min. A positive result was inferred by detection of a 500 bp band representing part of the meca gene by electrophoresis on a 1% agarose.

**Results**

Among 138 blood samples from neonates, 44 were positive blood culture for Staphylococci spp, that 24 (54.5%) and 20 (45.5%) were Coagulase negative Staphylococci and Staphylococcus aureus respectively. (Table1). Total 24 samples of CONS were biotyped. S. epidermidis 15 (62.5%) was the most common species followed by S. saprophyticus 9 (37.5%).

Table 1. Abundance distribution of Staphylococci spp from the neonate ward at Beasat hospital, Sanandaj, Iran

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>Number of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>24</td>
<td>54.5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>20</td>
<td>45.5</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Percentage of resistance of isolated Staphylococci spp from the neonate ward at Beasat hospital, Sanandaj, Iran

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>CoNS Rate of resistance (%)</th>
<th>S. aureus Rate of resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazole-trimethoprim</td>
<td>34.1</td>
<td>22.7</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>29.5</td>
<td>34.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>36.3</td>
<td>34.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>29.5</td>
<td>36.3</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>18.1</td>
<td>20.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8.3</td>
<td>15.0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>25.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>62.5</td>
<td>75.0</td>
</tr>
</tbody>
</table>

**Discussion**

Bacterial pathogens particularly Staphylococci spp pose a significant threat to human health generally neonates. (Shubhra .S,2009) In current study, from 138 blood cultures, 44 (31.9%) Staphylococci spp were isolated and identified, which 24 (54.5%) and 20 (45.5%) were Coagulase negative Staphylococci (CONS) and Staphylococcus aureus respectively. Many studies from elsewhere in the world still report that CONS are the most common organisms associated with neonatal sepsis(AlFaleh,2010) . In previous study Alfaleh (AlFaleh,2010) 55.11% and gheib (Gheibi,2008)54% CONS isolated which is comparable to present study, while in study of Iran , kalantar et al 65.78% CONS reported(Kalantar,2007). Antimicrobial sensitivity pattern differs in different studies as well as at different times in Iran and overseas studies(Rahbar,2005, Yadegar,2009). In this study the most common resistance in staphylococci spp was to ampicillin that About 75% for S.aureus and 65% for CONS, so also 80% S.aureus were resistant to Oxacillin. In study of torret S. aureus resistance to ampicillin was 85%(Yano,2009) and in Philippine, rate of Oxacillin-resistant S. aureus from clinical specimens was 66 % (Arakama,2010). Moreover, because of widespread methicillin resistance among Staphylococci spp, the most frequent causative microorganism among neonates, and empiric treatment of Staphylococcal infection with vancomycin is advocated strongly in many neonatal wards(Kalantar, Motlagh,2007, Gheib.S 2008). In our study, we observed that more than 20% resistance to vancomycin in S.aureus and in CONS 18%. Saderi reported Prevalence of Staphylococcus strains resistant to vancomycin in iran (Shahrbanooie,2005).
In Britain, France, the United state reports of outbreak strains VRSA observed (Tenover FC, 2001).

In our study, prevalence methicillin resistant rate in MRCONS was 55.6% that lower than from other previous reports done in other countries such as Turkey (74.4%), France (71%) (Khadri and Alzohairy, 2010), and Iran 70% (Davoodi, 2012). On the other hand, in this study, prevalence of MRSA was 55%. According to statistics in the United States, the prevalence of MRSA was 2% in 1980 and in 2004 was 60% (Lin Y, 2007). Frequency of MRSA in Asian countries such as India 44% (Kupfer, 2010), Saudi Arabia was 8% (Broens, 2011), and Iran 56% (21).

The high prevalence of MRSA and MRCONS isolates in Iran can be due insufficient infection control measures in hospitals and inappropriate use of methicillin. We tested all isolated *Staphyloccocus spp* for detection of mecA gene. The highest mecA gene carriage was found in CONS strains. 21 (87%) of 24 CONS strains from the neonates were mecA positive (Figure 1). Disk diffusion method could not recognized total staph strains that have mecA gene. One of the most reliable method for the identification of meticiline resistance is detection of mec A gene by PCR (Velasco, 2005). In this study, 20 samples of *S.aureuse*, 14 strain were positive for mecA gene and 16 strain were meticiline resistance. kolbert observed similar cases in CONS Despite the lack of mecA gene were meticiline resistance (Kolbert, 1995). This can due be production high amounts B-lactamase in strains.

CONS predominated as the cause of meticiline resistance in our study. Our results shown that disk diffusion method is not an accurate method for the determination of meticiline susceptibility for *staphyloccocus spp* and a Rapid method as PCR for antibiotic susceptibility is important to institute appropriate therapy.

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