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 Staphylococci*(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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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). Staphylococci 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 B_-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 neonate 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 CTTTGGTCTTTCTGCATTCCTG.

Amplification was done using a thermal regime of 35 cycles of amplification at 95^{0}C for 5 min, and 95^{0}C for 45s which was for denaturation. Annealing temperature was set at 58 ^{0}C for 45s. The extension phase was done using a temperature of 72 ^{0}C for 1 min and the 2nd one was 72 ^{0}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%) .

The antibiotic susceptibility patterns of *Staphylococci* isolates are shown in Table(2). In Among *CONS a*nd *Staphylococcus aureus* 62.5% and 75% resistance was seen maximum with Ampicillin and minimum with Ciprofloxacin for both *CONS* and *S. aureus* which was 8.3% and 15 % respectively. Among 24 *CONS*, 19 (79.1%) were meticiline resistance(MR) and among 20 *S. aureus* strains, 16 (80%) were MR by routine disc diffusion test using Oxacillin disc. prevalence of *MRCONS* was 55.6% and *MRSA* was 55%. Screening for *mecA* gene by using PCR method revealed that 21 (87%.) and 14 (70%) strains of *CONS a*nd *S. aureus* were positive for *mecA* gene respectively.

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

Isolated bacteria	Number of isolates	Percentage
Coagulase-negative staphylococci	24	54.5
S. aureus	20	45.5
Total	44	100

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

Antibiotic	*CoNS*	*S. aureus*
	Rate of resistance (%)	
Sulfamethoxazole-trimethoprim	34.1	22.7
Gentamicin	29.5	34.0
Erythromycin	36.3	34.0
Tetracycline	29.5	36.3
Vancomycin	18.1	20.4
Ciprofloxacin	8.3	15.0
Clindamycin	25.0	30.0
Ampicillin	62.5	75.0

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* S*taphylococci* (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 ghieb (Gheibi,2008)54% *CONS* isolated which is comparable to present study, while in study of Iran , kalantar *et.a*l 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 ampicilin that About 75% for *S.aureuse* and 65% for *CONS* , also 80% *S.aureus* were resistant to Oxacillin. In study of torret *S. aureuse* resistance to ampicilin 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).

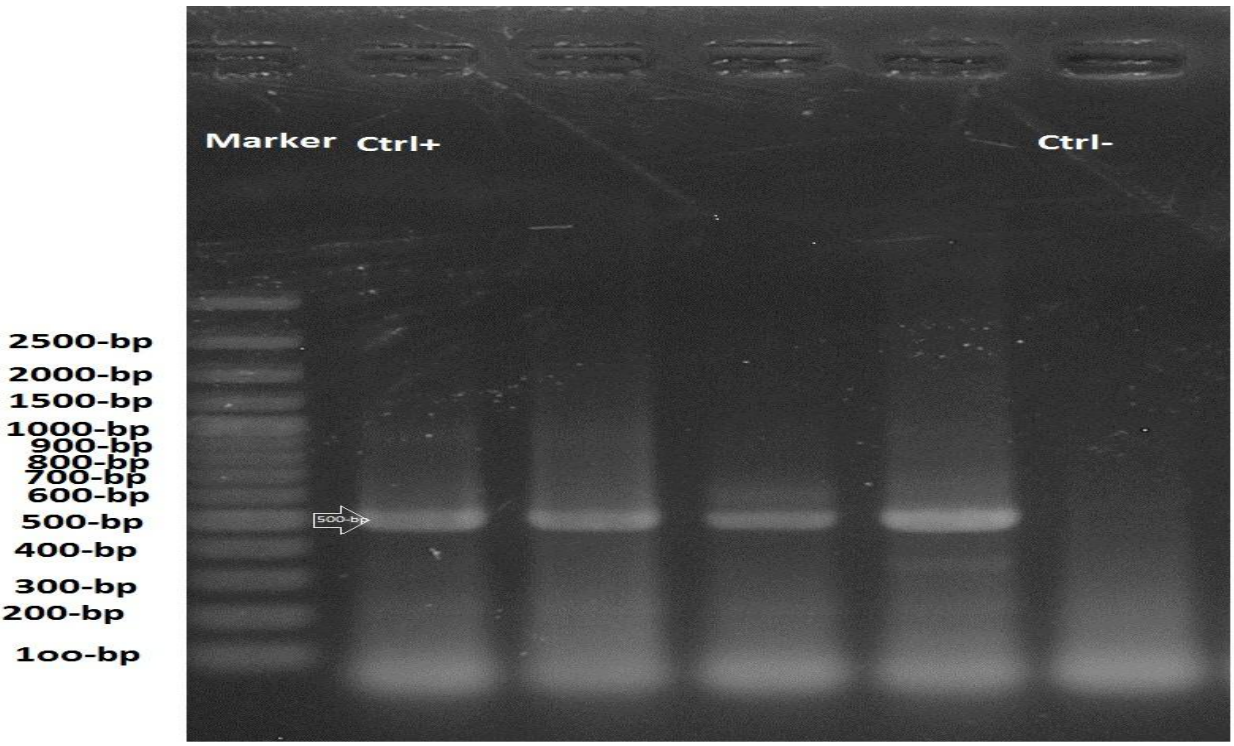

Figure 1. PCR analysis for the *mec*A gene among *Staphylococci spp* isolated from the neonate ward at Beasat hospital, Sanandaj, Iran. Line 1, DNA marker; line 2, 3,4 and 5 have *mec*A gene; Line 6 negative control, lacking *mec*A gene.

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 iran56%(21).

The high prevalence of *MRSA* and *MRCONS* isolates in Iran can be due insufficient infection control measures in hospitals and inappropriate use of methiciilin. We tested all isolated *Staphylococcus 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 *mec A* 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 methicillin resistance in our study. Our results shown that disk diffusion method is not an accurate method for the determination of methicillin susceptibility for *staphylococci spp* and a Rapid method as PCR for antibiotic susceptibility is important to institute appropriate therapy.

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