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ORIGINAL ARTICLE

MIC trends of vancomycin and teicoplanin among methicillin resistant CoNS isolates from new born blood cultures in a tertiary care centre in Southern India

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ABSTRACT

Background and Objectives: Coagulase-negative-Staphylococci (CoNS) are important etiological agent of bacteraemia in newborn babies. Methicillin resistant CoNS infections have only limited treatment options. The antimicrobial susceptibility pattern of Methicillin Resistant CoNS isolates from newborn blood cultures was studied with special reference to MICs of Vancomycin and Teicoplanin.

Materials and Methods: The study population included Methicillin Resistant Coagulase Negative Staphylococcal isolates (MRCoNS) from newborn blood cultures, during a one-year period. Minimum inhibitory concentration (MIC) of Vancomycin and Teicoplanin in Methicillin resistant CoNS isolates was determined by macrobroth dilution method as per CLSI guidelines and by automated methods.

Results: Coagulase Negative Staphylococci were the etiological agent in 73.7% (n=56) cases of neonatal bacteremia. Methicillin resistance in newborn CoNS was found to be 58.9%. All the MRCoNS isolates had vancomycin and teicoplanin MICs in the susceptible range. There were MRCoNS isolates with MICs in the upper limit of susceptible range for both vancomycin and teicoplanin, which can result in poor clinical response.

Conclusion: Continuous large scale multi-centre surveillance studies with special attention to study the MIC pattern of the high-end anti-MRSA agents like vancomycin, teicoplanin, linezolid are to be carried out. This will help the clinicians to judiciously prescribe the antibiotics, which is very essential for antimicrobial stewardship.

Keywords: Staphylococci; Sepsis; Vancomycin; Teicoplanin; Minimum inhibitory concentration

INTRODUCTION

Coagulase-negative-Staphylococci (CoNS) are an important etiological agent of bacteraemia and sepsis in the new born babies. Approximately 20% of very low birth weight preterm infants experience late onset neonatal sepsis. Half of these infections are caused by coagulase negative Staphylococci and are associated with a mortality rate of 9%. *S. epidermidis* accounts for 60-93% of the infections caused by coagulase negative Staphylococci, with lesser contributions by *S. haemolyticus*, *S. hominis*, *S. warneri*, *S. saprophyticus*, *S. cohni* and *S. capitis* (1). Even though mortality rates are less, CoNS infections in the neonate can significantly increase the morbidity by increasing the number of days of hospitalisation. Recently, CoNS

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have been noted as a prominent cause in early onset sepsis also, in very-low-birth –weight infants.

Methicillin resistance is a significant problem among Coagulase-negative-Staphylococci. The prevalence of Methicillin resistance in CoNS varies from 30-60% (2-4). The treatment options for MRCoNS infections are limited to agents such as vancomycin, linezolid and teicoplanin. It was in the mid-1980s that the first clinical isolates of *S. epidermidis* with reduced susceptibility to glycopeptides were described. Though vancomycin resistance is not a major problem in India, recent reports from southern and northern parts of India have documented the emergence of vancomycin intermediate and resistant strains among staphylococcal isolates (4). Reports on teicoplanin susceptibility among CoNS are scarce from India. CoNS resistance to glycopeptides applies exclusively to teicoplanin although the mechanisms involved are not clear.

The MIC (Minimal Inhibitory Concentration) of a bacterium to a certain antimicrobial agent gives a quantitative estimate of its susceptibility to the antibiotic. MIC is defined as the lowest concentration of antimicrobial agent required to inhibit the growth of the organism. The rising MICs of vancomycin among Vancomycin susceptible *S. aureus* is referred to as vancomycin MIC creep. In patients with *S. aureus* bacteremia, higher vancomycin minimum inhibitory concentrations (MICs) have been associated with prolonged bacteremia or increased mortality (5). Vancomycin creep has been observed in both Methicillin sensitive and Methicillin resistant staphylococcal isolates (5). Among coagulase negative Staphylococci, it is commonly observed with *S. haemolyticus* and *S. epidermidis* (6).

With the ever-increasing number of immunocompromised patients, prolonged hospital stays, frequent interventions and use of intravascular devices, coagulase negative staphylococci are emerging as important pathogens capable of causing life-threatening infections. Alarming rise in methicillin resistance in *S. aureus* and other coagulase negative staphylococci is limiting the utility of all beta lactam agents, thus considerably limiting the therapeutic options. The appearance of strains of MRSA with raised MICs and clinical resistance to vancomycin, linezolid and teicoplanin is a cause for concern. The use of vancomycin to treat infections caused by MRSA having vancomycin MIC 2 µg/ml also needs caution since therapeutic efficacy of vancomycin in such situation may not be rewarding (7, 8).

In our institution, Coagulase negative Staphylococci is the most common isolate obtained from new born blood cultures. Therefore, a study was undertaken to note the susceptibility patterns of Methicillin Resistant CoNS isolates from our new born blood cultures to vancomycin and teicoplanin by determining their MICs by two methods. This study will therefore help us to know the susceptibility/ resistance pattern and MIC trends of MRCoNS isolates in our institution to vancomycin and teicoplanin.

MATERIALS AND METHODS

The study was conducted in Clinical Microbiology Lab in a tertiary care centre in Southern India. The study population included the Methicillin Resistant Coagulase Negative Staphylococcal isolates (MR-CoNS) isolated within 24 hours of incubation from new-born blood cultures during a period of one year. If more than one MRCoNS isolate was obtained from a patient, only one isolate was included in the study.

The colony morphology, Gram stain, catalase test and absence of coagulase enzyme were used to identify CoNS. Methicillin resistance was determined using different CLSI-approved methods (cefoxitin disk diffusion, oxacillin MIC by automated method) for the different coagulase negative staphylococci (9). The BD Phoenix automated Microbiology system was used for the identification of the species and antibiotic susceptibility testing of the MRCoNS isolates. Minimum inhibitory concentration (MIC) of vancomycin and teicoplanin in Methicillin resistant CoNS isolates was also determined by the macrobroth (tube) dilution method as per CLSI guidelines (10). Vancomycin powder was procured from Sigma Aldrich (India), teicoplanin powder and CA-MHB (media) were obtained from HiMedia (India).

Tube dilution method to determine MIC was performed and interpreted as per the recommendations of the Clinical and Laboratory Standards Institute (CLSI), which classify CoNS strains as 'susceptible', 'intermediate', or 'resistant' as follows: For Teicoplanin- MIC values of ≤8 µg/l, 16 µg/l and ≥32 µg/l, respectively and for Vancomycin- MIC values of ≤4 µg/l, 8 to 16 µg/l and ≥32 µg/l respectively. *Staphylococcus aureus* ATCC 29213 was used as the quality control in each set of tests. The dilutions tested were 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 µg/l. The procedure

was performed in duplicate on separate occasions.

We thus analysed the MICs of vancomycin and teicoplanin in the MRCoNS isolates obtained by two methods - tube dilution and automated Phoenix BD System. The data obtained was numerically coded and entered in Microsoft Excel spread sheet. Further statistical analysis was done using the SPSS software.

RESULTS

During the study period, a total of 2577 blood specimens were received for culture and sensitivity and 368 of them were from new born babies. Out of these 368 neonatal blood cultures, 76 yielded positive results. Therefore, the blood culture positivity rate for the new born was 20.7 during the period.

The most common isolate obtained from the neonatal blood culture was Coagulase negative Staphylococci (CoNS) (Fig. 1). There were 56 CoNS isolates, out of which 33 were Methicillin Resistant. Coagulase Negative Staphylococci were the etiological agent in 73.7% (56/76) cases of neonatal bacteremia in our institution. The percentage of Methicillin resistance in new born CoNS was found to be 58.9% (33/56) (Fig. 2). Therefore, MRCoNS constituted 43.4% (33/76) cases of neonatal bacteremia. The species distribution of the MRCoNS isolates is given in Table 1. The susceptibility pattern of the MRCoNS isolates is given in Fig. 3.

All the 33 MRCoNS isolates had vancomycin and teicoplanin MICs in the susceptible range- that is for Vancomycin, all the isolates had MIC ≤4 µg/ml and for teicoplanin, all the isolates had MIC ≤8 µg/ml.

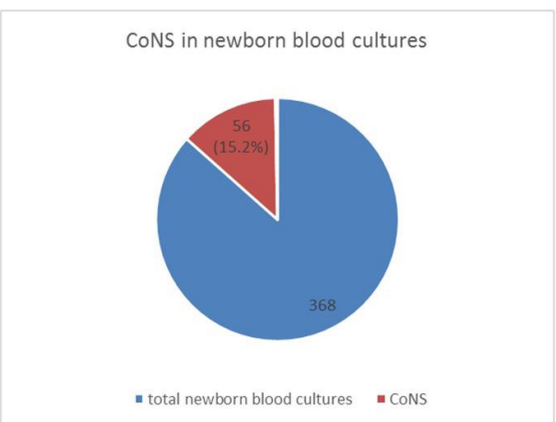

Fig. 1. Percentage of CoNS isolates from new born blood cultures

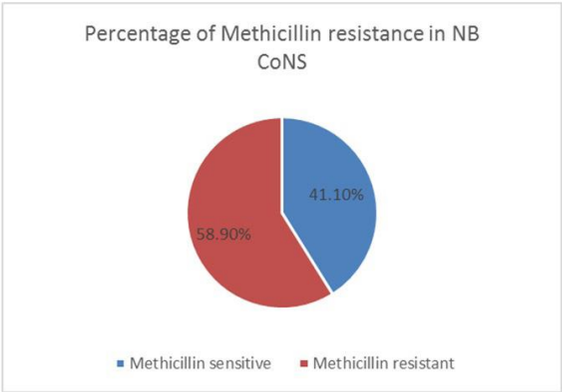

Fig. 2. Percentage of Methicillin resistance in new born CoNS isolates

Table 1. Species of CoNS isolated

SL. No.	Species	Number
1	*S. epidermidis*	11
2	*S. hemolyticus*	10
3	*S. saprophyticus*	4
4	*S. lentus*	2
5	*S. auricularis*	1
6	*S. capitis*	1
7	*S. equorum*	1
8	*S. gallinarum*	1
9	*S. kloosi*	1
10	*S. xylosus*	1
	Total	33

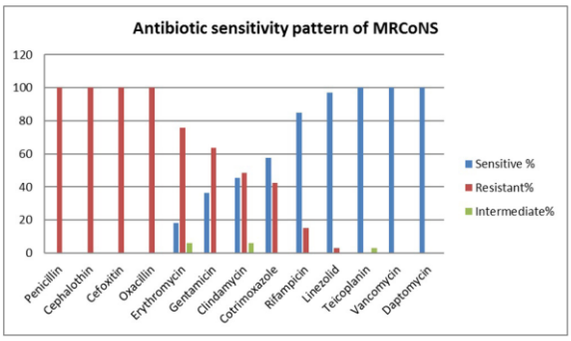

Fig. 3. Antibiotic Susceptibility Pattern of MRCoNS isolates

The MIC ranges of vancomycin and teicoplanin in the isolates are depicted in Tables 2-4. The MICs of vancomycin ranged from 0.25 µg/ml to 2 µg/ml by tube dilution and from 1 µg/ml to 2 µg/ml by automated method. The MICs of teicoplanin ranged from 0.25 µg/ml to 2 µg/ml by tube dilution and from 1 µg/ml to

Table 2. Vancomycin and Teicoplanin MIC of MRCoNS from new born

MIC value of Vancomycin (µg/ml)		No: of MRCoNS with Vancomycin MIC (Phoenix BD)	No: of MRCoNS with Vancomycin MIC (Tube dilution)	MIC value of Teicoplanin (µg/ml)		No: of MRCoNS with Teicoplanin MIC (Phoenix BD)	No: of MRCoNS with Teicoplanin MIC (Tube dilution)
Susceptible	<1	0	16	Susceptible	<1	0	12
	1	25	13		1	11	10
	2	8	4		2	14	11
	4	0	0		4	7	0
Intermediate	8	0	0		8	1	0
	16	0	0	Intermediate	16	0	0
Resistant	32	0	0	Resistant	32	0	0

Table 3. Vancomycin MIC of different species of MRCoNS from new born

Species/Method	No. Of isolates with Vancomycin MIC			
	<1	1	2	≥4
S. epidermidis				
Broth dilution	6	5	0	0
Automated	0	9	2	0
S. hemolyticus				
Broth dilution	3	5	2	0
Automated	0	4	6	0
S. saprophyticus				
Broth dilution	1	3	0	0
Automated	0	4	0	0
S. lentus				
Broth dilution	0	1	1	0
Automated	0	2	0	0
S. auricularis				
Broth dilution	0	0	1	0
Automated	0	1	0	0
S. capitis				
Broth dilution	1	0	0	0
Automated	0	1	0	0
S. equorum				
Broth dilution	1	0	0	0
Automated	0	1	0	0
S. gallinarum				
Broth dilution	1	0	0	0
Automated	0	1	0	0
S. kloosi				
Broth dilution	0	1	0	0
Automated	0	1	0	0
S. xylosus				
Broth dilution	0	1	0	0
Automated	0	1	0	0
Total isolates				
Broth dilution	13	16	4	0
Automated	0	25	8	0

Table 4. Teicoplanin MIC of different species of MRCoNS from new born

Species/Method	No. Of isolates with Teicoplanin MIC			
	<1	1	2	≥4
S. epidermidis				
Broth dilution	5	6	0	0
Automated	0	1	9	1
S. hemolyticus				
Broth dilution	0	3	7	0
Automated	0	0	4	6
S. saprophyticus				
Broth dilution	3	0	1	0
Automated	0	4	0	0
S. lentus				
Broth dilution	1	0	1	0
Automated	0	2	0	0
S. auricularis				
Broth dilution	0	0	1	0
Automated	0	1	0	0
S. capitis				
Broth dilution	1	0	0	0
Automated	0	1	0	0
S. equorum				
Broth dilution	1	0	0	0
Automated	0	1	0	0
S. gallinarum				
Broth dilution	0	0	1	0
Automated	0	0	0	1
S. kloosi				
Broth dilution	1	0	0	0
Automated	0	1	0	0
S. xylosus				
Broth dilution	0	1	0	0
Automated	0	0	1	0
Total isolates				
Broth dilution	12	10	11	0
Automated	0	11	14	8

8 µg/ml by automated method. No discrepancies were found in duplicates done by tube dilution.

DISCUSSION

Neonatal sepsis is defined as sepsis developing in the first 28 days of life or that developing within 4 weeks of expected date of confinement in preterm babies (11). Prematurity and infections together account for the greatest burden of neonatal deaths (12). Coagulase negative Staphylococci have been found as a major pathogen of neonatal sepsis especially late onset sepsis in the preterm. One important reason for this may be the interventions that are done to save even the most preterm neonates.

The most common isolate obtained from neonatal blood cultures during the study period from our institution was coagulase negative Staphylococci. The percentage of CoNS isolates from new born blood cultures was 15.2% (56/368) and out of the 76-culture positive neonatal blood cultures, 56 was due to Coagulase negative Staphylococci (73.7%). Seventy-three percent of all neonatal bacteremia in the United States is caused by CoNS pathogens (13, 14). Determining the clinical significance of coagulase negative Staphylococci isolated from blood cultures is difficult, particularly in neonates where we often receive only single specimens for culture. In general, a time to positivity of less than 24 hours is considered consistent with true bacteremia. So, we included only those CoNS isolates from new born cultures which grew within the first 24 hours.

Methicillin resistance was found in 58.9% of the isolates in our study. Methicillin resistance ranging from 50-90% have been noted in various studies (15-17). Phenotypic expression of methicillin (oxacillin) resistance in CoNS is much more heterotypic than that observed in *S. aureus* meaning the percentage of population that expresses high level oxacillin resistance is smaller. Regardless of the heterotypy observed, all isolates with methicillin resistance are resistant to all beta lactam antibiotics. Therefore, a major population of the CoNS isolates are found to be multi-drug resistant including the beta lactam agents.

The antibiotic susceptibility pattern of the MRCoNS isolates in our study showed 100% resistance to cefoxitin and all beta lactam agents, 75.8% resistance to erythromycin, 63.6% resistance to gentamicin, 48.6% resistance to clindamycin, 42.4% resistance to cotrimoxazole, 15.2% resistance to rifampicin but was 97% susceptible to linezolid and 100% susceptible to vancomycin, teicoplanin and daptomycin. The increased rate of methicillin resistance very frequently limits the treatment options to high end antibiotics like vancomycin, teicoplanin, linezolid and daptomycin.

In the last two decades, a worldwide increase in the number of CoNS with decreased susceptibility to glycopeptides has been described. Therefore, a precise determination of MICs of these antibiotics has gained significance. Hence, we determined MIC of vancomycin and teicoplanin of the MRCoNS isolates from new born blood cultures by two methods- broth macro dilution (tube dilution) and automation (Phoenix BD). We found that the MICs determined by the two methods for all the MRCoNS isolates were in the susceptible range for both vancomycin and teicoplanin. Several studies have reported uniform susceptibility of CoNS to vancomycin and teicoplanin, as is the case in our study.

However, there are many reports of decreased vancomycin and teicoplanin susceptibility among Staphylococci, which has made a precise determination of Vancomycin and teicoplanin MIC very important (18, 19). Broth macro dilution is a method recommended by CLSI but is labour intensive and tedious to perform whereas automated method (Phoenix BD) is easier to perform but expensive in resource poor settings. The MICs of vancomycin of the MRCoNS isolates in our study ranged from 0.25 µg/ml to 2 µg/ml by tube dilution and from 1 to 2 µg/ml by automated method. The MICs of teicoplanin ranged from 0.25 µg/ml to 2 µg/ml by tube dilution and from 1 µg/ml to 8 µg/ml by automated method. It was noted that MICs obtained by automated method were 1 to 2-fold higher than that obtained by broth macro dilution.

Several studies have reported that staphylococcal isolates with MIC ≥ 2 µg/ml have decreased clinical response (20, 21). In our centre, eight MRCoNS isolates (24%) showed an MIC of 2 µg/ml by automated method, whereas four MRCoNS isolates (12%) showed an MIC of 2 µg/ml by tube dilution method. Many studies have reported a rise in vancomycin MICs over time, a phenomenon also known as vancomycin 'MIC creep'. But in studies carried out in the setting of low vancomycin use, no changes in vancomycin MICs over certain time were detected.

Yet another challenge in this regard is the variable MIC results obtained by different methods. Therefore, large-scale multi-centre standardised studies are the need of the hour to evaluate the MIC trends of staphylococcal isolates to vancomycin in our country.

Resistance to teicoplanin is commoner in MR-CoNS in contrast to that in MRSA where it is rare. But regional differences definitely exist in the teicoplanin resistance rates. Teicoplanin MICs of 8, 4 and 2 respectively were found in 1, 7 and 14 MRCoNS isolates in this study, by automated method. By tube dilution method, there were no MRCoNS isolates with teicoplanin MIC 8 µg/ml or 4 µg/ml, but 11 isolates had a teicoplanin MIC of 2 µg/ml. Most of these isolates with higher MICs belonged to *Staphyloccus haemolyticus* spp. In a study from Brazil on clinical isolates of *Staphylococcus aureus* and coagulase negative staphylococci, it was observed that teicoplanin 800 mg every 24 hours can achieve >90% target attainment for isolates with MICs upto 1 µg/ml, but if the MIC of teicoplanin increases to 2 µg/ml, the rate of target attainment decreases to about 50% (22).

There are certain limitations for our study. The study was conducted in a tertiary care centre in Central Kerala (Southern India) with a limited number of study samples and also for a short duration. MIC trends of the high-end antibiotics like vancomycin, teicoplanin and linezolid and the phenomenon of MIC creep can be better derived if continuous large-scale nation-wide surveillance studies are planned and worked out. We also didn't do a clinical follow up to find out the clinical response in the patients especially in those with raised vancomycin and teicoplanin MICs but still in the susceptible range.

To conclude, with our study we found that CoNS was the most common etiological agent of neonatal bacteremia in our institution showing a Methicillin resistance of about 58.9%. All the MRCoNS isolates were susceptible to vancomycin and teicoplanin. But there were a small proportion of isolates with raised MICs for vancomycin and teicoplanin, even though they were still in the susceptible range. Continuous large scale multi-centre surveillance studies with special attention to study the MIC pattern of the high-end anti-MRSA agents like vancomycin, teicoplanin, linezolid are to be carried out, which can help the clinicians to judiciously prescribe the antibiotics, which is very essential for antimicrobial stewardship.

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