Methicillin resistant coagulase negative *Staphylococcus*: From colonizer to a pathogen

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Abstract: The objective of our study was to determine the frequency of methicillin resistance in coagulase negative Staphylococcus (CoNS) and to determine its in-vitro antimicrobial susceptibility to various other routinely used antibiotics. It was a cross sectional study conducted at the department of Microbiology, Army Medical College, Rawalpindi, Pakistan from June 2011 to May 2012. The organisms were identified on the basis of colony morphology, Gram staining, catalase, DNAase and slide/tube coagulase tests. The organisms were considered to be methicillin resistant when the diameter of zone of inhibition was less than 25mm around 30µg cefoxitin disc. Antibiotic sensitivity was determined using the Modified Kirby-Bauer disc diffusion method. From a total of 337 CoNS, 201 were methicillin resistant and were included in the study. All were resistant to Penicillin, followed by Erythromycin (93·1%), Ciprofloxacin (77%), Co-trimoxazole (74·8%), Gentamicin (68·3%), Clindamycin (51·06%), Tetracycline (44·6%), Fusidic acid (40%), Rifampicin (39·5%), Chloramphenicol (19·3%), Linezolid (2%), Minocycline (1·1%), and Vancomycin (0%). More than half of CoNS were methicillin resistant. Vancomycin is the only drug to which all of the MRCoNS were sensitive, with more than 98% of the isolates being sensitive to Linezolid and Minocycline.

Keywords: Methicillin, antibiotic resistance, methicillin resistant coagulase negative *Staphylococcus.*

INTRODUCTION

Once given little importance and usually considered as culture contaminant or part of the normal flora of skin, oral and nasal mucosa (Ibrahem *et al*., 2009), coagulase negative Staphylococcus (CoNS) is now confronting us and demanding respect which was its due. Methicillin resistant coagulase negative Staphylococcus (MRCoNS) is becoming a source of growing concern. One of the reasons is the ability of CoNS to form biofilms on foreign bodies (such as prosthetic heart valves, prosthetic joints and intravenous catheters) as well as on native structures such as heart valves (John and Harvin, 2007; Carvera *et al*., 2009). Biofilms on plastic tubings, which have become very common in our hospitals protect bacteria from both antibiotics (Galdbart, 2000; Rupp and Archer, 1994; Wisplinghoff *et al*., 2003) and host immune defenses i.e. antibodies and neutrophils (Warren Levinson 11th Edition). Health care workers can serve as a source of infection by CoNS for the immunocompromised patients (Ibrahem *et al*., 2009). With superadded ever increasing methicillin resistance treatment of infection by MRCoNS is getting even more complex.

MRCoNS are becoming resistant to most of the antibiotics in clinical use. Multi resistance in CoNS is carried on a Staphylococcal chromosome cassette (SCC) which almost always includes the *mec*A gene for resistance to semi-synthetic penicillins (SCC*mec*) (Hanssen, 2004). Thus a MRCoNS may simultaneously show resistance to many antibiotics (Archer, 1991). CoNS showing decreased susceptibility to Vancomycin have also been reported (Garrett, 1999; John and Harvin, 2007). In addition MRCoNS probably serve as a source of Methicillin resistance gene for MRSA (Barbiers, 2010; Wielders, 2001). Resistance to other antibiotics may also be transferred from MRCoNS with the SCC*mec* to MRSA (Archer and Johnston, 1983; McDonnell *et al*., 1983; Forbes and Schaberg, 1983).

Encountering increased isolation of MRCoNS over the years, we set out to investigate the prevailing situation with regards to MRCoNS in our setup.

Objective

To determine the frequency of Methicillin resistance in CoNS and to determine the *in vitro* antimicrobial susceptibility to various others routinely used antibiotics.

MATERIAL AND METHODS

Study design

Cross sectional study

Place of study

Department of Microbiology, Army Medical College (National University of Sciences and Technology Islamabad)-Pakistan

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Duration of study
June-2011 to May-2012

Sample selection
Non-probability convenience sampling. Staphylococci isolated from various clinical specimens were included. The specimen site (blood, pus, body fluid, sputum, wound swab) was recorded.

Inclusion criteria
Coagulase negative *Staphylococcus* resistant to methicillin.

Exclusion criteria
Methicillin sensitive coagulase negative *Staphylococcus* Duplicate samples of same patients. *Staphylococcus aureus*

Sample handling
The specimens were inoculated onto Blood, Chocolate and MacConkey agar plates and incubated at 35°C for up to 48hrs. Blood samples were inoculated in Brain Heart Infusion broth (BHI) for 24 hours at 35°C before sub culturing onto the agar plates.

The organisms were identified on the basis of colony morphology, Gram staining, catalase and tube coagulase tests. Gram-positive cocci in clusters giving a positive catalase test were identified as Staphylococci. Of these, those showing positive slide coagulase were *S. aureus* and those showing negative reaction were subjected to tube coagulase test. A one in six dilution of plasma in saline (0.85% NaCl) was made. A colony of test organism was emulsified in 1ml of diluted plasma and incubated at 37°C. The tubes were examined at 1, 2 and 4 hours for clot formation. Tubes with no clot were left at room temperature overnight. Next day tubes with any degree of clot formation were taken as *S. aureus* and tubes with no clot were taken as CoNS (Baird, 14th Edition).

Saline suspension of the colonies of CoNS equivalent to 0.5 McFarlands turbidity standard was prepared. Antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) methods (CLSI, 2012). Isolates were subjected to sensitivity test against commonly used anti-staphylococcal antimicrobials like Penicillin (10 units), Erythromycin (15ug), Clindamycin (2ug), Tetracycline (30ug), Gentamicin (10ug), Minocycline (30ug), Ciprofloxacin (5ug), Chloramphenicol (30ug), Linezolid (30ug), Rifampicin (5ug), Fusidic acid (10ug) and Vancomycin (30ug). A 30µg Cefoxitin disc was used to determine Methicillin resistance. Antimicrobial discs (Oxoid) were applied and incubated at 37°C for 24 hours.

Next day the sensitivity plates were examined using transmitted light for growth (fig. 1). The isolates were reported as Methicillin sensitive coagulase negative Staphylococcus (MSCoNS) or MRCoNS based upon the diameter of the zone of inhibition around the Cefoxitin (30ug) disk. The organism was considered to be Cefoxitin resistant when the diameter of the zone of inhibition was less than 25mm. (as per CLSI criterion).

RESULTS

During this study period 337 CoNS were isolated. Of these 201 (59.64%) were resistant to Methicillin by virtue of a zone of inhibition smaller than 25mm around a 30ug Cefoxitin disk. The antibiotic susceptibility pattern of these 201 isolates was noted. Majority of the clinical specimens revealing Methicillin resistant coagulase negative *Staphylococci* were from blood (120), followed by pus swabs (35), double lumen tips (15), urine (12), high vaginal swabs (6), eye swab (2), nasal swabs (2), Sputum (2), nasobronchial lavage (2), ear swab (1), throat swab (1), pleural fluid (1) and cerebrospinal fluid (1). This distribution is summarized in table 1.

Fig. 1: Meuller Hinton agar plate with antibiotic discs showing zone of inhibition

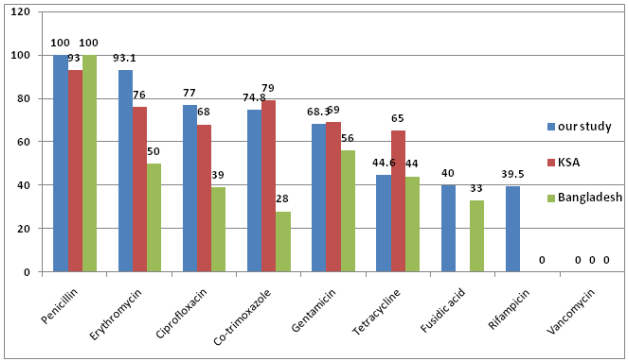

Fig. 2: Comparison of resistance of different antibiotics

All the 201 MRCoNS strains were uniformly resistant to Penicillin, followed by Erythromycin (93.1%), Ciprofloxacin (77%), Cotrimoxazole (74.8%), Gentamicin (68.3%), Clindamycin (51.06%), Tetracycline

(44.6%), Fusidic acid (40%) and Rifampicin (39.5%). Less resistance rate was observed against Chloramphenicol (19.3%) Linezolid (2%) and Minocycline (1.1%). All MRCoNS strains tested in this study were uniformly sensitive to Vancomycin (100%).

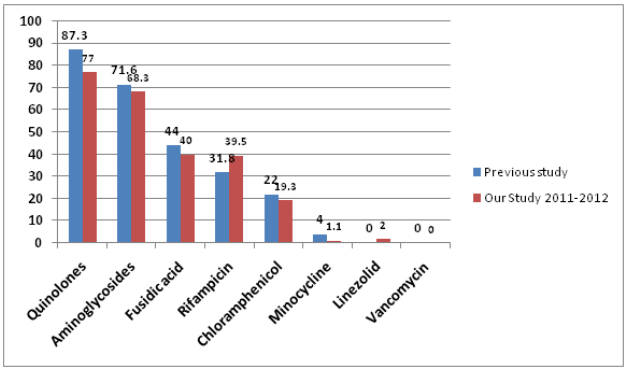

Fig. 3: Change in Antibiogram with time

The antimicrobial resistance pattern of MRCoNS isolates against agents of different classes is summarized in table 2.

Table 1: Disrtibution of MRCoNS in various specimens (n=201)

Specimen	Percentage
Blood	120
Pus	35
Tips	15
Urine	12
High vaginal swabs	6
Eye swabs	2
Nasal swabs	2
Sputum	2
Nasobronchial lavage	2
Ear swab	1
Throat swab	1
Pleural fluid	1
Cerebrospinal fluid	1

DISCUSSION

The rates of Methicillin resistance have increased considerably and 60-85% strains are resistant to Methicillin according to different studies (Cuevas *et al*., 2004; Keuhnert, 2006; Miragaia, 2005). With increasing Methicillin resistance these organisms are also becoming resistant to most of the other antibiotics in use. So the detection of MRCoNS in health-care settings has never been more important due to increasing frequency of MRCoNS over the years, the limited therapeutic choices available and because these might be source of genes of Methicillin resistance in MRSA.

Our study showed that Methicillin resistance rate was 59.64%. Other studies conducted in different countries show rates to be 74.4% in Turkey (Khadri and Alzohairy, 2010); 71% in France (Khadri and Alzohairy, 2010); 67.4% in Germany (Sader, 2007; Koksal, 2007); 56.25% in Bangladesh (Haque *et al*., 2010) and 39.4% in isolates from India tested in Kingdom of Saudi Arabia (Khadri and Alzohairy, 2010). Our results are close to those of Bangladesh.

Table 2: Resistance pattern of MRCoNS against various antibiotics (n=201)

Antibiotic	Percentage of resistant MRCoNS (%)
Penicillin	100
Erythromycin	93.1
Ciprofloxacin	77
Co-trimoxazole	74.8
Gentamicin	68.3
Clindamycin	51.06
Tetracycline	44.6
Fusidic acid	40
Rifampicin	39.5
Chloramphenicol	19.3
Linezolid	2
Minocycline	1.1
Vancomycin	0

In our setup rates of methicillin resistance have increased from 22.7%, 34.3% and 56.64% (June 2009 to May 2012) (Latif *et al*., 2015). Previously rates of methicillin resistance carried out in another study in our department from 2008-2010 was 40.1% (Shah *et al*., 2014)

MRCoNS are emerging as increasingly resistant organisms with complete resistance to penicillin, high rate of resistance to erythromycin, ciprofloxacin, co-trimoxazole, gentamicin, clindamycin. All of our isolates were sensitive to vancomycin and high susceptibility rates were observed against minocycline and linezolid.

In a study conducted by Khadri & Alzohairy in KSA in 2007 (Khadri and Alzohairy, 2010), the resistance rates of different antibiotics was (93%) being resistant to penicillin, followed by co-trimoxazole (79%), erythromycin (76%), cephalexin (69%), gentamicin (69%), ciprofloxacin (68%), and tetracycline (65%).

When compared with the study in Bangladesh (Haque *et al*., 2010) resistance to Penicillin, Amoxycillin, Oxacillin and Cloxacillin was 100% followed by gentamicin (56%), erythromycin (50%), doxycycline (44%), cephradine (44%), ciprofloxacin (39%), fusidic acid (33%), cefuroxime (33%) and ceftriaxone (28%). All isolates of MRSE were susceptible to Rifampicin and Vancomycin.

Previously another study in our department found the rates of resistance to various antibiotics to be Quinolones (87.3%), Aminoglycosides (71.6%), Fusidic acid (44%), Rifampicin (31.8%), Chloramphenicol (21.9%), Minocycline (4%), Linezolid (0%), Vancomycin (0%). The results are compared in fig. 3.

CONCLUSION

More than half (59.64%) of our coagulase negative Staphylococci were methicillin resistant. Most of these MRCoNS showed considerable resistance to routinely used anti-Staphylococcal antibiotics. Vancomycin is the only antibiotic to which all the isolates were sensitive. More than 98% of the isolates were sensitive to Linezolid and Minocycline. We need to carefully monitor MRCoNS as they are fast evolving into pathogens, treatment options for which are already very limited and may become even narrower.

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