ORIGINAL ARTICLE

Coagulase negative staphylococci — a fast emerging threat

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Abstract

Objective: To determine the frequency of isolation of coagulase-negative staphylococci and their resistance to methicillin over a period of time.

Methods: The descriptive cross-sectional study was carried out at Army Medical College, Rawalpindi, from June 2009 to May 2012, and comprised clinical samples mostly from patients admitted to the intensive care unit. They were inoculated onto appropriate culture media depending upon the specimen. After 24-hour incubation at 35°C, coagulase-negative staphylococci were identified on the basis of colony morphology, gram staining, a positive catalase and a negative tube coagulase test.Methicillin resistance among the isolated staphylococci was determined using a 30µg Cefoxitin disc as per the Clinical and Laboratory Standards Institute protocol. Number of coagulase-negative staphylococci for each year and their methicillin resistance rates were calculated. A comparison was made with methicillin resistant staphylococcus aureus) isolated during the same period.

Results: Of the total 1331 specimens studies over three years, 581(43.65%) were coagulase-negative staphylococci. The rate of coagulase-negative staphylococci and methicillin resistance was higher each year; 110(26.6%) in May 2009-Jun 2010, 134(36.5%) in 2011, and 337(61%) in 2012. Methicillin resistance rates also increased from 25(22.7%) to 46(34.3%) and then to 201(59.6%) in 2012.Maximum isolated specimens came from blood 311(53.5%), followed by pus/swabs 204(35.1%).

Conclusion: The frequency of isolation of coagulase-negative staphylococci and its methicillin resistance among hospitalised patients is on the rise.

Keywords: CoNS, Methicillin, MRCoNS. (JPMA 65: 283; 2015)

Introduction

Coagulase-negative staphylococci (CoNS) are one of the most frequently isolated bacteria in a microbiology laboratory. Notorious for being considered as cultural contaminants or part of normal skin or mucosal flora, recent advancements in medical technology, especially in prosthetic devices, have proposed a major challenge for the microbiologists to distinguish the contaminant strains from clinically significant CoNS.[1] They are now increasingly being reported as a cause of bacteraemia in immunocompromised and hospitalised patients, especially with indwelling medical devices.[2] Among CoNS, 50% to 70% catheter-related bloodstream infections are caused by Staphylococcus epidermidis.[1,2] Other less frequently isolated CoNS include staphylococcus saprophyticus, staphylococcus lugdunensis, staphylococcus haemolyticus and staphylococcus schleiferi.[1]

Penicillins are the mainstay of treatment for staphylococcal infections but with more improved formulations being generated over the years the mechanisms for acquiring resistance in staphylococci have also changed. The beta lactamase production by staphylococcus aureus was counteracted with the discovery of methicillin, a beta lactam stable penicillin in 1960s, but soon methicillin resistant staphylococcus aureus (MRSA) emerged.[1,3] Molecular studies have shown that this resistance is mediated by mec-A gene located on mobile genetic elements called staphylococcal cassette chromosome (SCCmec), and its eight different types (I-VIII) have been discovered so far.[3]

With increasing rates of isolation of CoNS over the years, their resistance to methicillin is also increasing. This is because methicillin resistant coagulase-negative staphylococci (MRCoNS) colonise the skin of healthcare workers and hospitalised patients.[4] This colonisation also act as a reservoir for isolates that are multi-drug resistant and also serve as a source of antibiotic resistant genes which can be transferred among CoNS as well as to staphylococcus aureus.[4] These are also difficult-to-treat infections because of the ability of these bacteria to form biofilms,thus rendering difficulty in antibiotics to penetrate them.[1] The CoNS are already being blamed for the transfer of mec-A gene to staphylococcus aureus in vivo, therefore its increasing frequency worldwide is a matter of serious concern.[5]

The increased isolation rates of CoNS and MRCoNS in

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various clinical samples along with its increasing pathogenicity prompted this study to assess its frequency in our facility.

Material and Methods

The cross-sectional study was carried out at the Department of Microbiology, Army Medical College, Rawalpindi, National University of Sciences & Technology, Islamabad, Pakistan from June 2009 to May 2012.

All the clinically significant samples of patients received in the laboratory for culture and sensitivity were collected by simple random technique irrespective of age and gender. These patients had indwelling devices or were admitted in Intensive Care Unit (ICU) of Military Hospital, Rawalpindi, which is an 1100-bed tertiary care facility.

Duplicate samples from the same patient received during same course of illness were excluded. The studied specimens included blood, pus, wound swabs, body fluids, urine, catheter/double lumen tips and sputum. Blood, pus/swabs and catheter tips were inoculated onto blood and MacConkey agar, urine on cysteine electrolyte deficient agar and sputum on to blood and chocolate agar plates. Blood samples were incubated in Brain Heart Infusion broth for 24 hours at 35°C prior to subculture on blood and MacConkey agar plates. After 24-hour incubation at 35°C aerobically, the organisms were identified on the basis of colony morphology, gram staining, catalase and tube coagulase tests. Small grey white (usually non-haemolytic) colonies on blood agar plates and lactose fermenting colonies on MacConkey agar plates, showing gram-positive cocci in grape like clusters on gram stain and a positive catalase test was identified as staphylococci. A negative tube coagulase test was used to identify the CoNS. A saline suspension equivalent to 0.5 McFarlands turbidity standard for each isolate was prepared by mixing similar-looking colonies in 2ml of saline. The suspension was then inoculated onto Muller Hinton agar plate and a 30µg cefoxitin disc was applied for detecting methicillin resistance as per the Clinical and Laboratory Standard Institute (CLSI) protocol.[13] After 24-hour incubation at 35°C aerobically, isolates showing zone of inhibition ≥25mm around the cefoxitin disc were identified as sensitive whereas isolates showing zone of inhibition of ≤24mm were identified as MRCoNS.

Number of CoNS for each study year was calculated, and their resistance to methicillin and sample distribution was determined. Data was analysed using SPSS 17 and was presented graphically using Microsoft power point and Microsoft word. Methicillin resistance rates among Staphylococcus aureus (MRSA) isolated during the same time period were also compared with those of MRCoNS.

Results

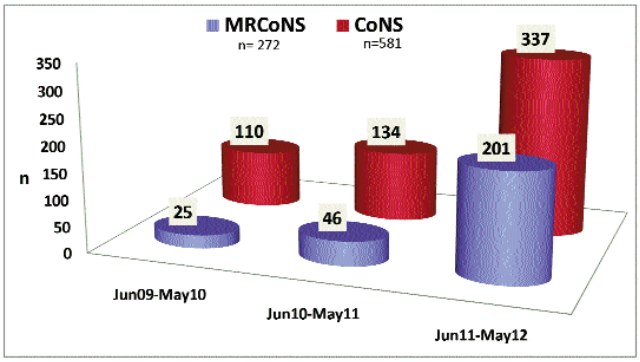
Of the total 1331 specimens studies over three years, 581(43.65%) were CoNS. The rate of CoNS was higher each year; 110(26.6%)in May 2009-Jun 2010, 134(36.5%) in 2011, and 337(61%) in 2012 (Table-1). Methicillin resistance (MRCoNS) rates also increased from 25(22.7%) to 46(34.3%) and then to 201(59.6%) in 2012 (Figure-1).

Out of the 581 CoNS isolated, 311(53.5%) came from blood specimens, followed by 204(35.1%) from pus/swabs (Table-2). The rise in MRCoNS isolation rates was much marked

Table-1: Frequency of isolation of CoNS from Jun09-May12.

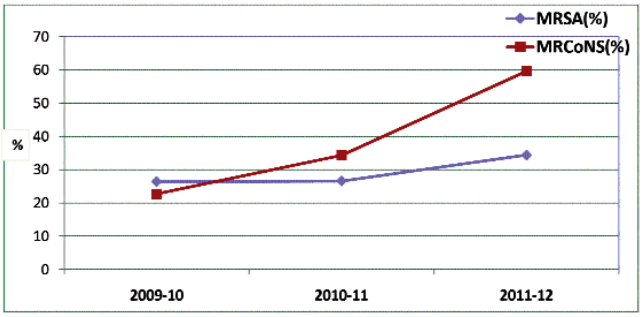
Year	Number of Staphylococci (n= 1331)	Number of CoNS (n= 581)	% of CoNS
Jun 2009- May 2010	412	110	26.6
Jun 2010- May 2011	367	134	36.5
Jun 2011-May 2012	552	337	61.0

CoNS: Coagulase-negative staphylococci.



CoNS: Coagulase-negative staphylococci

Figure-1: Methicillin resistance rates among CoNS (MRCoNS)Jun09-May12.



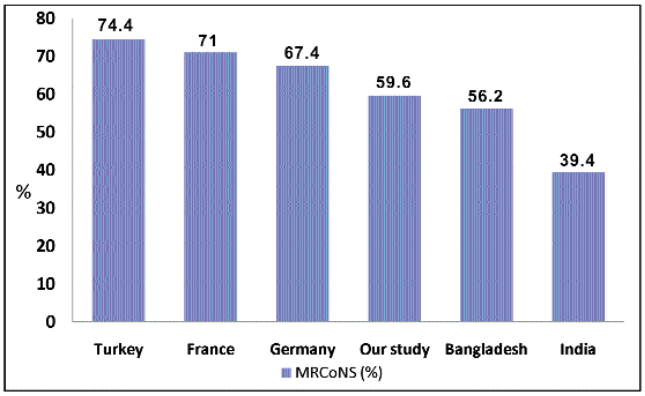
MRSA: Methicillin resistantstaphylococcus aureus
MRCoNS: Methicillin resistant coagulase negative staphylococci

Figure-2: Isolation rates of MRSA and MRCoNS from Jun09-May12.

Table-2: Sample distribution among CoNS (Jun09-May12).

S. No.	Sample	Number of CoNS (n=581)	% of CoNS
1.	Blood	311	53.5
2.	Pus, swab, tips	204	35.1
3.	Urine	60	10.3
4.	Sputum	06	01

CoNS: Coagulase negative staphylococci.



MRCoNS: Methicillin resistant coagulase negative staphylococci.

Figure-3: MRCoNS Prevalence in other countries.

when compared with MRSA isolated during the same time period (Figure-2).

Device of delusion to confusion

Discussion

Methicillin resistance in CoNS due to mec-Agene renders them resistant to all the β-lactams, leaving us with few antibiotics for treatment.[6] Among CoNS about 70% to 80% of clinical isolates reported are resistant to methicillin.[1] The increasing rate of isolation of CoNS and MRCoNS observed in our study correlates with national and international data available. A study showed prevalence of CoNS and MRCoNS among various clinical samples as ranging between 30% and 43%.[7] Prevalence of MRCoNS was found to be 70% while determining antimicrobial susceptibility pattern among CoNS isolates from Civil Hospital Karachi.[8]

Prevalence of MRCoNS in Turkey, France, Germany, Bangladesh and Kingdom of Saudi Arabia also range between 39.4% and 74.4% (Figure-3).[9-12]

According to the National Nosocomial Infectious Surveillance of the United States of America (NNIS), the rates of methicillin resistance among staphylococci have increased in the last two decades.[13] In five-point prevalence studies in Spain, the methicillin resistance rates among CoNS increased from 32% in 1986 to 61.3% in 2002.[14]

The NNIS has also reported that the incidence of CoNS increased from 9%-27% and methicillin resistance among CoNS increased from 20% to 60% from 1980 to 1989 while NNIS report from January 1992 to June 2004 showed 88.4% of nosocomial infections caused by MRCoNS.[13] The NNIS and Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) programmes rank CoNS as leading cause of nosocomial blood stream infections while SENTRY programme rank them as second most common cause. When considering combined nosocomial and community-acquired bacteraemia, they are considered third most common cause by NNIS and SCOPE. The rateof methicillin resistance among CoNS as determined by these surveillance programmes are almost consistent with each other; 77.3% by NNIS, 80.4% by SCOPE and 75% by Intensive Care Antimicrobial Resistance Epidemiology.[13,15,16]

Various recent studies also show increasing isolation rates of CoNS in blood stream infections. A study from a tertiary care hospital in Tanzania reported CoNS as the most common pathogen (67.4%) isolated from the blood samples of patients[17] while in England they are reported to be the second most common pathogen (16.9%) responsible for causing bacteraemia.[18] The blood samples received from various Canadian tertiary care hospitals ranked CoNS as the third most common pathogen (11%).[19] In our study CoNS constituted 20% of blood-borne pathogens isolated from 2009-2012. While determining antimicrobial resistance pattern among gram-positive cocci in China the prevalence of MRCoNS was found to be 89.5%[20] study found that colonisation with MRCoNS increased from 20%-47% five days post-operatively in patients undergoing major abdominal studies.[21]

Although not a part of our study in addition to bacteraemia prosthetic valve endocarditis (PVE) and native valve endocarditis (NVE) also has an important association with CoNS. It is believed that more than 10% of all cases of infective endocarditis are caused by CoNS and they are the most common pathogens causing intracardiac prosthetic device infections (PVE, pacemaker and cardiac defibrillator lead endocarditis). In a prospective cohort study, 16% of non-intravenous drug users with PVE have been found to be associated with CoNS and 67% of these isolates were methicillin resistant staphylococcus epidermidis (MRSE).[22] In case of native valve endocarditis in non-intravenous drug users 8% have been caused by CoNS with 41% being MRSE.[23]

As mentioned, the increasing rate of isolation of MRCoNS is alarming because of its possibility of transferring the mec-

Agene to staphylococcus aureusin vivo.[5] Various studies support this idea. One study determined the MRCoNS carriage rate among community to be 19.2% and that a strong structural homology existed between SCC-IVa in MRSE and MRSA.[24] Nasal carriage of MRCoNS was determined in a point prevalence study carried out in Finland after an outbreak with MRSA (SCCmec V). This study revealed that 61% of the residents harboured MRCoNS and 3% carried both MRSA and MRSE which shared the same SCCmectype V.[25]

Conclusion

The frequency of isolation of CoNS and its methicillin resistance is on the rise to the extent that it is almost doubling each year.Hence, they may emerge as a substantial challenge for healthcare systems if ignored. Maintaining adequate antisepsis and decontamination guidelines, avoiding prolonged use of indwelling medical devices, limiting injudicious use of antibiotics and further evaluation of MRCoNS epidemiology is the need of the hour.

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