MEDICAL JOURNAL ARMED FORCES INDIA XXX (2016) XXX–XXX

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevier.com/locate/mjafi

Short Communication

Prevalence and molecular characterization of methicillin resistance among Coagulase-negative Staphylococci at a tertiary care center

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ARTICLE INFO

Article history:
Received 8 October 2014
Accepted 10 March 2016
Available online xxx

Keywords:
Methicillin-resistant Coagulase-negative Staphylococci (MR-CoNS)
Cefoxitin disk
*mec*A gene
Polymerase chain reaction (PCR)

ABSTRACT

Background: Methicillin-resistant Coagulase-negative Staphylococci (MR-CoNS) have emerged as an important cause of nosocomial infections especially in patients with prosthetic devices and implants. This study was conducted with an aim to determine the prevalence of methicillin resistance among CoNS isolates at a tertiary care center by both phenotypic and genotypic methods.

Methods: This cross sectional study was carried out from September 2011 to February 2014 in which 150 non-repetitive clinical isolates of CoNS were identified at the species level by conventional phenotypic methods. Cefoxitin disk (30 μg) diffusion testing was used to determine methicillin resistance and confirmed by detection of *mec*A gene by polymerase chain reaction (PCR).

Results: Out of 150 CoNS isolates, 51 were methicillin resistant by cefoxitin disk diffusion method. Out of these 51 isolates, *mec*A gene was detected only in 45 isolates. Moreover, *mec*A gene was also detected in 4 isolates, which were cefoxitin sensitive. Thus, the prevalence of methicillin resistance among CoNS was found to be 32.7% by PCR.

Conclusion: The prevalence of methicillin resistance among Coagulase-negative Staphylococci (CoNS) was 32.7% by PCR detection of *mec*A gene. The sensitivity and specificity of cefoxitin disk diffusion method against *mec*A gene detection by PCR were found to be more than 90%. It can be concluded from this study that cefoxitin disk diffusion test can be used as a useful screening method to detect methicillin resistance among CoNS isolates. However, detection of *mec*A gene by PCR remains a more accurate method of detecting methicillin resistance among CoNS.

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http://dx.doi.org/10.1016/j.mjafi.2016.03.007
0377-1237/© 2016 Published by Elsevier B.V. on behalf of Director General, Armed Forces Medical Services.

Please cite this article in press as: Bhatt P, et al. Prevalence and molecular characterization of methicillin resistance among Coagulase-negative Staphylococci at a tertiary care center, *Med J Armed Forces India*. (2016), http://dx.doi.org/10.1016/j.mjafi.2016.03.007

Introduction

Coagulase-negative staphylococci (CoNS) are one of the most commonly isolated organisms in the clinical microbiology laboratory. They were earlier considered to be clinically insignificant contaminants, when isolated from clinical specimens due to their ubiquitous nature.[1] The frequent isolation of CoNS from intravenous catheters, blood and other normally sterile body fluids presents a persistent interpretive challenge to clinical microbiologists. However, their role as significant pathogens following surgery and in patients with prosthetic devices has now been well established. The possible explanation for their current clinical importance and increased prevalence includes being commensals on the skin, selection pressure due to widespread use of broad-spectrum antibiotics in the hospital and their ability of adherence and formation of biofilms on the surfaces of vascular catheters and other medical devices.[1]

Emergence and increasing prevalence of methicillin-resistant Coagulase-negative Staphylococci (MR-CoNS) are a cause for concern. National Nosocomial Infection Survey data of USA revealed that from 1980 to 1989, the proportion of nosocomial CoNS resistant to methicillin, oxacillin, or nafcillin increased from 20% to 60%. Most of these MR-CoNS were also resistant to multiple antimicrobial agents.[2]

Staphylococcal chromosome cassette (SCC) *mec* is a resistance island present in the genome of methicillin-resistant isolates, where *mec* is the genetic element, that is responsible for resistance to methicillin.[2] *mecA* gene encodes for a particular penicillin-binding protein (PBP) called PBP2A, which has a low affinity for methicillin and most of the other β-lactam drugs and is thus responsible for the intrinsic resistance of these isolates to almost all β-lactams. Such multidrug-resistant strains are becoming a significant threat to public health.[2]

This study was conducted with an aim to determine the prevalence of methicillin resistance among CoNS isolates by both phenotypic and genotypic methods.

Material and methods

This cross sectional study was carried out from September 2011 to February 2014. A total of 150 non-repetitive clinical isolates of Coagulase-negative Staphylococci were isolated from various clinical specimens received in microbiology laboratory of a tertiary care centre. Isolates from blood samples and intravascular catheter tips were interpreted as being pathogenic, and not merely contaminants, by collecting paired blood cultures and simultaneous collection of blood sample from peripheral vein respectively. Same CoNS species isolated from urine more than once was interpreted as being pathogenic and not merely contaminant.

Identification of CoNS at species level

All CoNS isolates were identified at the species level by conventional phenotypic methods such as colony morphology, Gram's stain, catalase test, slide and tube coagulase test, susceptibility to novobiocin and polymyxin B, PYR test, acetoin production, ornithine decarboxylase test and fermentation of glucose, maltose, sucrose, mannitol, trehalose and mannose.

Identification of MR-CoNS by phenotypic method

Cefoxitin disc (30 μg) is used as a surrogate marker for prediction of mecA gene mediated resistance to oxacillin and is the preferred method of testing of methicillin resistant CoNS. All the isolates were subjected to Cefoxitin disc diffusion testing using a 30 μg cefoxitin disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on Muller–Hinton agar (MHA) plate. Plates were incubated at 37 °C for 24 h and zone diameters were measured. An inhibition zone diameter of ≤24 mm was reported as Methicillin resistant and ≥25 mm was reported as Methicillin sensitive.

Detection of mecA gene by polymerase chain reaction

All the CoNS isolates were subjected to polymerase chain reaction (PCR) for detection of mecA gene, which encodes the low-affinity penicillin-binding protein PBP 2A, the main factor responsible for the methicillin resistance.

DNA extraction

DNA extraction was done from all isolates of CoNS using the QIAamp DNA mini kits from QIAGEN, Germany. Manufacturer's instructions were followed for extracting DNA from the fresh cultures.

Primers

Primers were procured from Sigma–Aldrich Chemicals Pvt. Ltd. and are shown in Table 1.

PCR reaction mixtures

PCR reaction mixtures were prepared under laminar flow under strict precautions to prevent cross contamination. Amplification was carried out with the following thermal cycling profile: initial denaturation for 4 min at 94 °C, 35 cycles of amplification consisting of 1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C, with 7 min at 72 °C for the final extension.

Table 1 – Sequences of forward and reverse primers used for detection of *mecA* gene in MR-CoNS isolates.

Gene	Primer sequence	No of bases	nmol	Amplicon size
mec A	(F): 5′-GTAGAAATGACTGAACGTCCGATAA-3′	25	38.9	310 bp
	(R): 5′-CCAATTCCACATTGTTTCGGTCTAA-3′	25	28.9	

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