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Prevalence of MR-CoNS in Blood Stream Infection among Patients Attending a Tertiary Care Hospital in East India- A Retrospective Study

Sudipto Mandal[1*]

[1]Assistant Professor, Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah-711202, India

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***Corresponding author:** Sudipto Mandal
Assistant Professor, Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah-711202, India

Abstract **Original Research Article**

Methicillin resistant- coagulase negative staphylococci (MR-CoNS) associated infection is a mounting concern in healthcare settings now a day. MR-CoNS are one of the main infectious agents of the hospital acquired infection (HAI). Increasing rate of antimicrobial resistance against available antibiotics of MR-CoNS is a developing issue in low income or lower middle income countries. ***Aim*:** The aim of this study was to know the prevalence of MR-CoNS in blood stream infection (BSI) among patients attending a tertiary care cancer center using phenotypic and genotypic methods. ***Materials & Methods*:** This retrospective study was conducted over a period of 12 months (Year 2022-2023) in patients with BSI caused by Gram Positive Cocci (GPC). Screening of MR-CoNS from direct blood culture bottle was done by real time PCR targeting *Spa* and *MecA* gene. Identification and Methicillin resistance pattern was checked by Vitek-2 automated system. ***Results*:** A total of 180 blood culture positive samples were investigated of which n= 76 were MR-CoNS, n=13 were MRSA, n= 18 were MSSA and n= 73 were Non Staphylococcus sp. Among n=76 MR-CoNS (36.33%) *S. haemolyticus*, (30.26%) *S. epidermidis*, (23.68%) *S. hominis*, (3.94%) *S. warneri*, (2.63%) *S. saprophyticus*, (1.31%) *S. arlettae*, (1.31%) *S. cohnii* were reported. ***Conclusion*:** The occurrence rate of MR-CoNS was highest (42.22%) among the BSI with GPC. Most of the Methicillin Resistant-CoNS isolates showed high level of resistance against widely used antibiotics but all the isolates susceptible against vancomycin. Moreover, the in house developed real time PCR targeting *Spa* and *MecA* gene came out as a reliable and rapid molecular screening tool for the differentiation of MRSA, MSSA, MR-CoNS and Non staphylococcus species among GPC from direct blood culture bottle.

Keywords: Staphylococcus, Methicillin Resistant, Coagulase Negative, Nosocomial Infection.

INTRODUCTION

Coagulase negative staphylococci (CoNS) are clinically important species causing health-care associated infections. CoNS are basically the commensal community residing as skin flora and are among the abundant bacteria in the clinical specimens. CoNS become true pathogens in immune-suppressed patients [1]. Blood stream infections (BSI) caused by CoNS become more serious when they are identified as methicillin-resistant CoNS (MRCoNS). Mostly, CoNS involved in BSI are acquired during exposure to hospitals and health care settings. In our country India, approximately 20-40% clinical isolates belong to CoNS [2].

The term methicillin-resistant CoNS denotes those coagulase-negative staphylococci which show resistance to methicillin but now refers to a multidrug resistance group and are susceptible only to glycopeptide group of antibiotics such as vancomycin. Despite the technological advancement in health care facilities and the usage of new regime of drugs, these infections are difficult to manage [3]. The term methicillin-resistant CoNS denotes those coagulase-negative staphylococci which show resistance to methicillin but now considered as multidrug resistance group and are susceptible only to glycopeptide antibiotics such as vancomycin [4]. The outbreak events of hospital acquired MR-CoNS are mainly due to nosocomial transmission of those bacteria from patient to patient and from healthcare workers to patient and vice versa. The objective of this retrospective study was to evaluate the prevalence of MR-CoNS in BSI among patients attending a tertiary care hospital in eastern India.

MATERIALS AND METHODS

The present study conducted in a tertiary care hospital of Eastern –India. This retrospective study was

conducted over a period of 12 months (Year 2022-2023) in patients with BSI caused by Gram Positive Cocci (GPC). Author prior describe the purpose of the study to all the participant patients before collecting the samples. Blood samples were collected in blood culture bottles from all the patients with BSI and send to microbiology laboratory for further processing.

Phenotypic Screening

The specimens which showed positive culture growth in BACT-Alert system were further characterized by conventional methods including colony characteristics, Gram-staining, slide coagulase test, growth on mannitol salt agar and identification was performed using automated system VITEK-2. The AST pattern of all the confirmed CoNS were determined by VITEK-2.

Genotypic Screening

To test a positive blood culture, with suspected Gram positive cocci in clusters, an aliquot of the blood culture media is transferred into a kit based sample buffer tube for bacterial DNA extraction according to the manufacturer instruction. QIAamp BiOstic Bacteremia DNA Kit (Qiagen), Cat No. 12240-50 was used to extract the nucleic acid. An extracted DNA was tested for Realtime PCR using SYBR-green dye for the *S. aureus* and MRSA-specific primers (Staphylococcus aureus protein A (*spa*), The mecA gene is part of a 21- to 60-kb staphylococcal chromosome cassette *mec* (SCCmec), a mobile genetic element that may also contain genetic structures such as Tn554, pUB110, and pT181 which encode resistance to non-lactam antibiotics) used to amplify the genetic targets, if present. The assay also includes a positive and negative control, *S. aureus* ATCC 43300 for positive for mec or methicillin resistance gene and *S. aureus* ATCC 29213 spa or protein A gene). Targeted Gene primers sequence are shown in table 1. The reporting format of typing of GPC isolates beased on PCR results is shown in table 2.

Table 1: Oligonucleotide sequences used in this study

***Spa*_F**	**TAAAGACGATCCTTCGGTGAGC**
***Spa*_R**	**CAGCAGTAGTGCCGTTTGCTT**
***Mec*_F**	**TCCAGATTACAACTTCACCAGG**
***Mec*_R**	**CCACTTCATATCTTGTAACG**

Table 2: Reporting format of typing GPC isolates.

Target Gene	**Result of PCR**			
spa	Positive	Positive	Negative	Negative
mecA	Positive	Negative	Positive	Negative
Type	MRSA	MSSA	MR-CoNS	Non-staph sp

RESULTS

A small retrospective study conducted in a tertiary care hospital to analyze the spectrum of MR-CoNS in a one-year period. Total of n=180 blood culture positive samples were investigated of which n=76 were MR-CoNS, n=13 were MRSA, n=18 were MSSA and n=73 were non-staphylococcus sp (Fig.1). Among n=76 MR-CoNS (36.33%) *S. haemolyticus*, (30.26%) *S. epidermidis*, (23.68%) *S. hominis*, (3.94%) *S. warneri*, (2.63%) *S. saprophyticus*, (1.31%) *S. arlettae*, (1.31%) *S. cohnii* were reported (Fig.2).

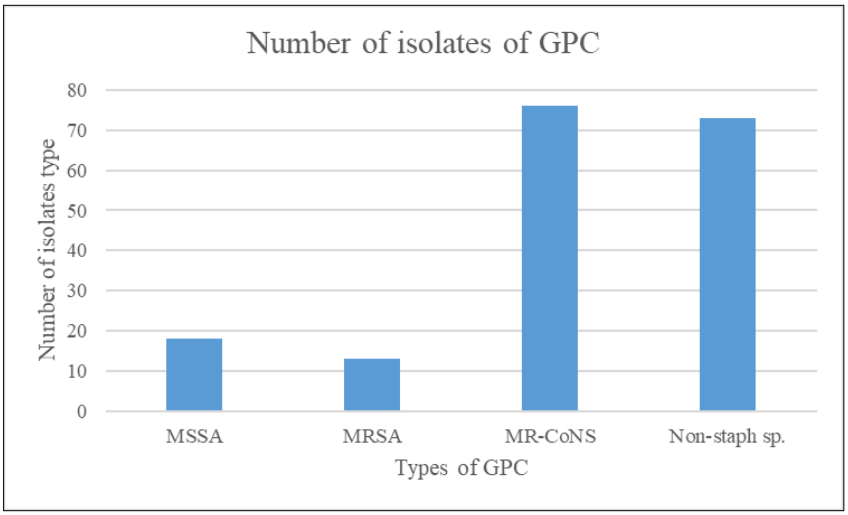

Fig. 1: Isolated types of Gram-positive cocci (GPC)

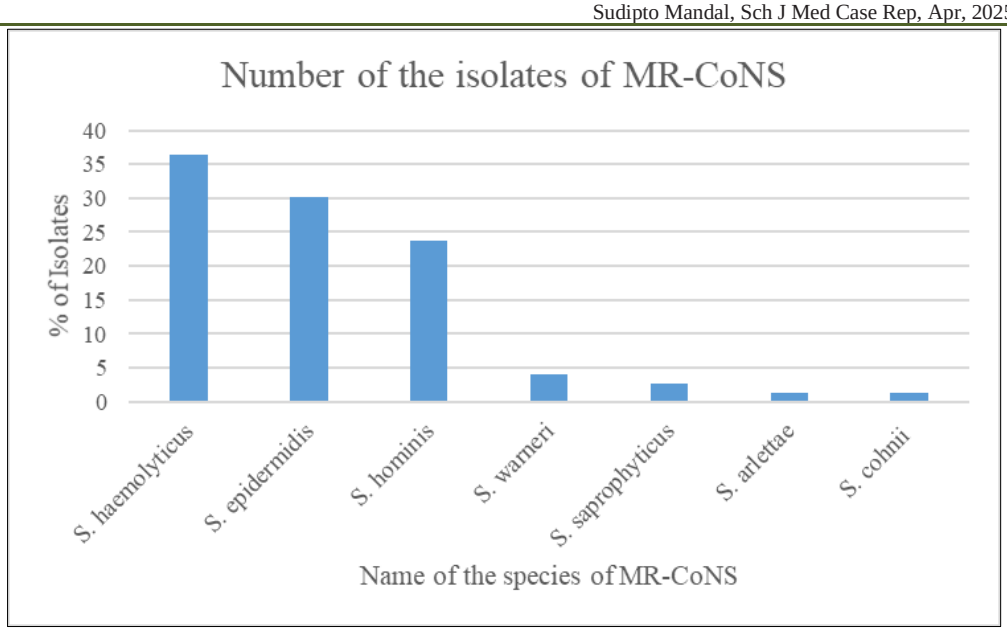

Fig. 2: Isolated species of Methicillin-resistant coagulase negative staphylococci (MR-CoNS)

CONCLUSION

This retrospective study revealed that the prevalence of MR-CoNS isolates was highest among the other Gram-positive Cocci (GPC) isolates. Among the MR-CoNS isolated (36.33%) *S. haemolyticus* and (30.26%) *S. epidermidis* contributed significantly to the resistance population against methicillin drug. However, all the isolates were found susceptible against vancomycin.

Moreover, the in house developed real time PCR targeting *Spa* and *MecA* gene came out as a reliable and rapid molecular screening tool for the differentiation of MRSA, MSSA, MR-CoNS and Non staphylococcus species among GPC from direct blood culture bottle.

Conflict of Interest: None

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