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Myron Anthony Godinho
Department of Biostatistics, MAHE, Manipal, India, g.sreejith@manipal.edu

Arjan Dhillon
Graduate of MAHE, g.sreejith@manipal.edu

Gan Kim Kun
Queen Elizabeth Hospital, Sabah, Malaysia, g.sreejith@manipal.edu

Shobana Raj
Queen Elizabeth Hospital, Sabah, Malaysia, g.sreejith@manipal.edu

Yong Kok Jen
Queen Eizabeth Hospital,Sabah, Malaysia, g.sreejith@manipal.edu

See next page for additional authors

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Nasal carriage of methicillin resistant Staphylococcus aureus (MRSA) among inpatients of a tertiary care centre: A cross-sectional study

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Original article

Nasal carriage of methicillin resistant Staphylococcus aureus (MRSA) among inpatients of a tertiary care centre: A cross-sectional study

Myron Anthony Godinho, Arjan Dhillon, Gan Kim Kun, Shobana Raj, Yong Kok Jen, Sreejith Govindan*

Email: g.sreejith@manipal.edu

Abstract

Context: Methicillin resistant *Staphylococcus aureus* (MRSA) is a nosocomial pathogen, which often exhibits multidrug resistance. It colonizes the anterior nares of humans, from where it can be transmitted to vulnerable individuals, causing life threatening infections. Using various phenotypic and genotypic methods, two types of strains have been identified such as hospital associated MRSA (HA-MRSA) and community associated MRSA (CA-MRSA). **Aims:** To evaluate the status of MRSA nasal colonization among inpatients of a tertiary care centre and classify them by antibiogram, Panton Valentine leucocidin (PVL) assay and staphylococcal cassette chromosome (SCC) mec typing. **Methodology:** A hospital based, cross-sectional study was conducted, involving 71 adult inpatients. Nasal swabs were collected and isolates were subjected to biochemical testing to identify *Staphylococcus aureus* (*S. aureus*). Antibiotic susceptibility testing, PVL assay, and SCC mec typing were also performed. Statistical analysis was performed using SPSS version 16. **Results:** Out of 71 nasal swab isolates, 11 were identified as MRSA. All the MRSA were resistant to ampicillin and ciprofloxacin. Erythromycin and clindamycin resistance were noticed in 88.9% and 44.4% of strains, respectively. Inducible clindamycin resistance was detected in two strains by D-Test. All isolates were negative for *pvl* gene. SCC mec typing revealed four Type-I strains, one Type-II strain and one Type-IV strain. The other five isolates could not be typed with the limited number of primers used in this study. **Conclusions:** Since majority of the MRSA were likely to be of HA-MRSA type, it is assumed that, the patients would have been colonized with them after hospitalization. This demands the necessity of MRSA screening for health care workers, which ultimately aids in planning strategies to control their outbreaks in health care settings.

Keywords: Nasal carriage, antibiotic resistance, CA-MRSA, HA-MRSA, MRSA, PVL, SCC mec type, surveillance

Introduction

Staphylococcus aureus (*S. aureus*) is a normal bacterial commensal in many humans, which usually colonizes the anterior nasal apertures. However, it may cause endogenous infection in the host itself or may get transmitted to susceptible individuals causing numerous life-threatening infections, including septicaemia, endocarditis, pneumonia, and necrotizing fasciitis. It may also complicate to toxic shock syndrome and scalded skin syndrome.

Myron Anthony Godinho
Research Associate, Department of Biostatistics, MAHE, Manipal, India

Arjan Dhillon
Independent Scholar, Graduate of MAHE

Gan Kim Kun
Medical House Officer, Queen Elizabeth Hospital, Sabah, Malaysia

Shobana Raj
Medical House Officer, Queen Elizabeth Hospital, Sabah, Malaysia

Yong Kok Jen
Medical House Officer, Queen Eizabeth Hospital,Sabah, Malaysia

Sreejith Govindan*
Associate Professor, Department of Microbiology, Melaka Manipal Medical College, MAHE, Manipal, India

*Corresponding Author
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Liberal and incomplete treatment of infectious diseases with penicillin, exerted selective pressures, which might have resulted in the emergence of penicillin resistant *S. aureus.* In 1961, a new drug resistant strain of *S. aureus* was emerged. This occurred due to the development of the *mecA* gene in the staphylococcal cassette chromosome (SCC), thus reducing *S. aureus*'s susceptibility to β-lactam antibiotics including methicillin and oxacillin. Such strains were capable of producing an altered penicillin binding protein (PBP2a) enabling the cell wall synthesis of such strains even in the presence of β-lactams drugs resulting the development of methicillin resistant *S. aureus* (MRSA).[1] Extensive genotypic analysis along with the phenotypic characterization helped to establish two distinct types of MRSA: Hospital associated MRSA (HA-MRSA) and community associated MRSA (CA-MRSA).[2]

HA-MRSA strains have been found to possess more drug resistance genes than CA-MRSA. This is likely due to their larger SCC mec genome, which helps them to accommodate more drug resistance genes. CA-MRSA strains are more virulent than HA-MRSA due to the production of Panton Valentine leukocidin (PVL) [3], which antagonizes leukocytes, causing necrotising pneumonia and necrotising fasciitis. Moreover, some *S. aureus* display inducible clindamycin resistance on exposure to macrolide-lincosamide-streptogramin (MLS) antibiotics like erythromycin[4], further restricting treatment options. Therefore, MRSA subtype identification becomes important for guiding antibiotic selection in such cases.

Identification and treatment of carrier states is essential to prevent transmission. The *S. aureus* nasal carrier state is determined by various factors and facilitates airborne distribution.[5,6] *S. aureus* colonizes 30-50% of adults and over 50% of children.[7,8] On the Indian subcontinent, overcrowding and unhygienic living conditions facilitate the colonization and transmission of MRSA worryingly; a national survey found that the intensive care unit (ICU) was most prone to MRSA colonization.[9] The present study investigates the scope of these issues at a tertiary care hospital in the Udupi district of Karnataka, India. The objectives of the study were to evaluate the MRSA nasal colonization among hospital inpatients and also to type the MRSA isolates based on antibiogram, PVL production, and SCC mec type.

Methodology

This study was conducted in 2011. This hospital based, cross sectional study recruited 71 willing, adult inpatients from a tertiary care centre at Udupi, Karnataka, India. Institutional ethics clearance committee approval was obtained prior to the study (IEC 173/2011). A convenient sampling approach was adopted. All the inpatients of medicine unit during a particular day were approached and the participant information sheet and the consent forms were given. Children and terminally ill patients were excluded. Except three patients, rest all patients provided the consent to participate in the study. The participants were educated on the importance of nasal colonization by *S. aureus* and informed regarding the procedure of sample collection. After obtaining their written informed consent, the subjects were recruited for the study.

A sterile cotton swab (Amie's charcoal swab, Hi-Media, Mumbai, India) was introduced 1cm into the subjects' anterior nares and brushed against the mucosa to collect the sample. Samples were inoculated on 5% sheep blood agar plates (Fi-Tech, Bangalore, India), labelled, and incubated aerobically at 37°C for 24-48 hours. Following incubation, golden yellow and other suspected colonies on blood agar were subjected to gram staining, catalase, and coagulase testing. Gram positive, catalase, and coagulase positive isolates were confirmed as *S. aureus* using the 'Hi Staph' latex agglutination test kit (Hi-Media, Mumbai, India). Five to six colonies from the culture plate were then subjected to cefoxitin (30μg) disc diffusion test for identification of MRSA. Following overnight incubation, a zone of inhibition (ZoI) of ≤21mm in diameter identified the isolate as MRSA. Antibiotic susceptibility testing (AST) was performed on all MRSA isolates using the Kirby Bauer disc diffusion method, as per clinical laboratory standards institute (CLSI) guidelines. ATCC 43300 MRSA standard strain was used for quality control. Ampicillin (AMP, 10μg), chloramphenicol (CHL,

30μg), ciprofloxacin (CIP, 5μg), erythromycin (ERY, 15μg), and clindamycin (CLI, 2μg) disks were included in AST.

Erythromycin resistant isolates underwent the 'D-test' to identify inducible clindamycin resistance.[4] An erythromycin disk (15μg) was placed 15mm from a clindamycin disk (2μg), on a lawn culture on Mueller Hinton agar. A 'D' shaped ZoI around the clindamycin disk, due to growth of bacteria in the areas of overlapping antibiotic diffusion, indicated inducible clindamycin resistance (Figure 1).

All MRSA isolates were further screened for *pvl* gene by PCR (Lina et al.).[10] A phenol chloroform method (In house developed protocol) was used for DNA extraction and PVL associated genes were amplified using Luk F PV and Luk S PV primers (Table 1). Thermal cycling (Nexus Gradient Mastercycler, Eppendorf) was performed with denaturation for five minutes at 95°C, followed by 30 cycles of denaturation (1 minute, 95°C), annealing (55°C, 1 minute) and extension (72°C, 2 minutes). A final extension for five minutes at 72°C was also done. The amplified products were subjected to gel electrophoresis at 125V for 25-30 minutes, before being analyzed under a UV transilluminator. The expected amplified product size was 433bp.

The MRSA strains were also subjected to SCC mec typing, by a multiplex PCR method as described by Milheirco et al. using primers specific to three regions of the staphylococcal housekeeping gene sequence, with the *mec A* gene as an internal control.[11] The primers (obtained from Sigma Aldrich) were specific to SCC mec type I, II, III, and IV only (table 1). USA300, NCTC 8325, and ATCC 43300 strains were used for quality control.

Table 1: Details of primers used in SCC mec typing

Loci	Primer	Product Size	SCC Type
A	5' TTCGAGTTGCTGATGAAGAAGG 3' 5' ATTTACCACAAGGACTACCAGC 3'	495	I
B	5' AATCATCTGCCATTGGTGATGC 3' 5' CGAATGAAGTGAAAGAAAGTGG 3'	284	II
C	5' ATCAAGACTTGCATTCAGGC 3' 5' GCGGTTTCAATTCACTTGTC 3'	209	II, III
D	5' CATCCTATGATAGCTTGGTC 3' 5' CTAAATCATAGCCATGACCG 3'	342	I, II, IV
Control	5' TCCAGATTACAACTTCACCAGG 3' 5' CCACTTCATATCTTGTAACG 3'	162	MRSA

SCC: Staphylococcus cassette chromosome

MRSA: Methicillin resistant Staphylococcus aureus

Results

Out of 71 samples collected, 54 (76.1%) grew coagulase negative staphylococci and were excluded from further testing. Coagulase positive *S. aureus* were isolated from the remaining 17 samples (23.9%). Among these, 11 (15.5%, 95%CI: 8.9-25.7) were identified as MRSA by cefoxitin disc diffusion method (figure 2). This finding was confirmed during PCR based SCC mec typing.

All isolates were resistant to ampicillin (AMP) and ciprofloxacin (CIP). Erythromycin (ERY) resistance was noticed in 88.9% of isolates and 44.4% were resistant to clindamycin (CLI), while another two, which displayed inducible clindamycin resistance (figure 3).

On PCR for *pvl* gene detection, there were no bands at the 433bp region, indicating that all 11 MRSA isolates were negative for the *pvl* gene (figure 4).

On SCC mec typing, all the MRSA strains displayed a 162bp band, confirming the presence of the *mecA* gene. The combined presence of other loci identified, four of the MRSA strains as SCC mec type I, one strain was SCC mec type II and another was type IV. The remaining five strains were unclassified due to the limited number of primers used in this study.

Discussion

A 1996 surveillance program of large hospitals in major Indian cities found that, up to 32% of *S. aureus* clinical isolates as methicillin resistant strains.[12] The occurrence of *S. aureus* colonization in resource poor settings has found to be lower in recent years.[13] In the present study, 15.5% (11 of 71) of cases were MRSA. It contributes to the 64.7% (95%CI: 49.3-82.7) of all *S. aureus* isolates. This is a worrying statistic because, being not a large Indian city by any comparison, 15.5% of MRSA colonization in this

study setting indicates the possibility of increased burden of MRSA in this area. This need to be explored using a more appropriate epidemiological study design with large sample size.

CA-MRSA has a smaller SCC and hence a smaller resistance profile, while HA-MRSA often displays resistance to antibiotics other than β-lactam groups, like fluoroquinolones and aminoglycosides. This is probably due to their large SCC cassette. High rates of multiple drug resistance in the present study suggested that most isolates were HA-MRSA; a finding corroborated by SCC mec typing, which confirmed five isolates as HA-MRSA (MRSA subtypes I and II) and only one as CA-MRSA (type IV). A high prevalence of CA-MRSA should have been expected, as subjects were hospitalized patients. The unclassified strains require further PCR based analysis with other primers to determine their subtype. On PVL assay, no isolates displayed *pvl* genes, which are one of the characteristic features of CA-MRSA. These findings are more reassuring when considering expected clinical implications, as highly virulent CA-MRSA has not yet been found in large volumes in our hospitals. However, these findings may not give the ultimate picture of the MRSA status of this geographical area as the sample size was limited. One need to keep in mind the overlapping of properties of HA and CA-MRSA, while interpreting the findings.

The presence of MRSA in health care setting environment is alarming, as inpatients are often immunocompromised due to their underlying conditions, such as extremes of age, infections, diabetes mellitus, malnutrition, or iatrogenic immunosuppression.[14] The antibiotic resistance pattern of the current study isolates suggests that they are probably the HA-MRSA type as they showed resistance to antibiotics other than β-lactam group. These patients were likely to be colonized after hospitalization, implicating the hospital staff as the most likely carriers. This indicates the need for screening the health care workers for nasal carriage of MRSA. An appropriate decolonization measures, such as the nasal application of mupirocin ointment is also essential in colonized case; though some studies report the emergence of mupirocin resistance in *S. aureus*.[15] Screening and decolonization have been shown to decrease the incidence of *S. aureus* infections in other Indian hospitals.[16] Redesigning ICU facilities and systems, reduces MRSA colonization and infection rates among these high risk patients.[17] Measures known to be effective include regular screening of hospital personnel, grouping infected patients by MRSA subtype, and isolating infected patients with select personnel who do not work with the uninfected.[18] The scope of screening hospital visitors needs to be evaluated, as they carry the risk of transmitting CA-MRSA, which may broaden its resistance profile in the antibiotic, rich hospital environment, creating a potential "superbug". However, screening cannot replace the regular infection control practices and should only be used in addition to that.[19] Alternative methods should also be sought, including "clean room" isolation and MRSA immunoprophylaxis. As the age old saying goes, "prevention is better than cure;" a strict quality surveillance system and highly motivated and dedicated infection control team can curb drug resistant pathogens such as MRSA in health care settings. One of the important limitations of this study was the convenient sampling approach. The small sample size could also have affected the power of the study.

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