Enferm Infecc Microbiol Clin. 2016;xxx(xx):xxx–xxx

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Original article

Predominance of SCC*mec* types IV and V among biofilm producing device-associated *Staphylococcus aureus* strains isolated from tertiary care hospitals in Mysuru, India

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ARTICLE INFO

Article history:
Received 15 February 2016
Accepted 7 September 2016
Available online xxx

Keywords:
Multi-drug resistance
Biofilm
Staphylococcus
MRSA
ica-dependent
ica-independent
spa-typing
SCC*mec*

ABSTRACT

Introduction: Device associated infections caused by *Staphylococcus aureus* in hospitalised patients is a serious healthcare problem. The present study was designed to determine the prevalence of biofilm-producing MRSA in device-associated infections.
Methods: Device-associated *S. aureus* strains ($n = 200$) obtained from two tertiary care hospitals in Mysuru city, India were screened for biofilm production, antibiotic resistance, Panton-Valentine Leucocidin genes, SCC*mec*-types, *spa*-types, and intercellular adhesion (*icaAD*) dependent and independent genes. The efficacy of antibiotics (linezolid, vancomycin and rifampicin) on biofilms was studied using MTT assay, and the results were correlated with the occurrence of *ica*-dependent and independent factors.
Results: Multidrug resistance was observed in 155 strains (77.5%), and 124 strains (62%) were identified as biofilm producers. Methicillin resistance was identified in 145 strains (72.5%), and SCC*mec* typing of these isolates revealed high prevalence of type IV and type V. They also showed increased prevalence of *pvl* gene. *icaAD* was identified in 65 isolates, with 37 isolates showing both *icaAD* and *ica*-independent genes. *spa* types t852 and t657 were predominantly observed in MRSA isolates. Those isolates that had both *ica*-dependent and *ica*-independent genes showed more resistance to the screened antibiotics than the *ica*-dependent alone.
Conclusion: This study reports a high prevalence of SCC*mec* type IV and V in biofilm producing *S. aureus* strains isolated from device-associated infections. Increased prevalence of *pvl* in SCC*mec* types IV and V strains suggests the role of community associated *S. aureus* in device-associated infections. The simultaneous presence of *ica*-dependent and independent genes increased the antibiotic resistance in established biofilms. Thus, *S. aureus* on medical devices is a potential risk for patients.

Predominio de SCC*mec* tipos IV y V en las cepas de *Staphylococcus aureus* productoras de biopelículas aisladas de infecciones de dispositivos médicos en hospitales terciarios de Mysuru, India

Palabras clave:
Multirresistencia antibiótica
Biopelícula
Staphylococcus
SARM
ica dependiente
ica independiente
tipos *spa*
SCC*mec*

RESUMEN

Introducción: Las infecciones asociadas a dispositivos médicos causadas por *Staphylococcus aureus* en pacientes hospitalizados son un problema importante. En el presente trabajo se estudia, en cepas de infecciones asociadas a dispositivos médicos, la prevalencia SARM productores de biopelículas y sus tipos SCC*mec*.
Métodos: Se usaron 200 cepas de *S. aureus* de infecciones de dispositivos médicos obtenidas de 2 hospitales terciarios de Mysuru, India. Se estudió la producción de biopelículas, los genes de la leucocidina de Panton-Valentine, los tipos SCC*mec*, los tipos de *spa* y los genes de adhesión intracelular (*icaAD*) dependientes e independientes. Se estudió la eficacia de linezolid, vancomicina y rifampicina en las biopelículas por un ensayo MTT y los resultados se correlacionaron con la presencia de genes *ica* dependientes e independientes.

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http://dx.doi.org/10.1016/j.eimc.2016.09.005

Please cite this article in press as: Halebeedu Prakash P, et al. Predominance of SCC*mec* types IV and V among biofilm producing device-associated *Staphylococcus aureus* strains isolated from tertiary care hospitals in Mysuru, India. Enferm Infecc Microbiol Clin. 2016. http://dx.doi.org/10.1016/j.eimc.2016.09.005

Resultados: Ciento veinticuatro cepas (62%) producían biopelículas y se observó multirresistencia antibiótica en 155 (77,5%). Eran resistentes a meticilina 145 cepas (72,5%) y en su tipificación SCC*mec* se observó alta prevalencia de los tipos IV y V. Estas cepas tenían una prevalencia superior de gen *pvl* a las no resistentes a meticilina. *icaAD* se identificó en 65 aislados, de los que 37 mostraron simultáneamente genes *ica* dependientes e independientes. Los spa tipos t852 y t657 se observaron predominantemente en las cepas de SARM. Los aislados que tenían a la vez genes *ica* dependientes e *ica* independientes presentaban mayor resistencia a los antibióticos probados que los que tenían solo *ica* dependientes.
Conclusión: El presente estudio informa de una alta prevalencia de SARM de los SCC*mec* tipos IV y V en cepas de *S. aureus* productoras de biopelículas. La elevada prevalencia del gen *pvl* en las cepas de los SCCmec IV y V sugiere el papel de los *S. aureus* comunitarios en las infecciones asociadas a estos dispositivos. La presencia simultánea de genes *ica* dependientes e independientes aumenta la resistencia a antibióticos en las biopelículas establecidas. Por todo ello, las cepas de *S. aureus* en dispositivos médicos son un riesgo potencial para los pacientes.

Introduction

Medical devices have been extensively used in health care; but they are known to promote bacterial adherence through biofilms. The contact of medical devices with the extracellular matrix proteins in patients, can trigger biofilm production and cause device-associated infections. *Staphylococcus aureus*, an important pathogen of community and hospital acquired infections, has been frequently reported to form biofilm on indwelling devices such as orthopaedic implants, urinary catheters, central venous catheters, peripheral venous catheters, endotracheal tubes, cardiac prosthetic valves, contact lens and on surgical sites.[1]

Biofilms formed by *S. aureus* on orthopaedic implants are reportedly known to cause increased morbidity in patients when compared to the infection on other implants.[2] But *S. aureus* biofilm on peripheral venous catheters (PVC) and central venous catheters (CVC) is also a major problem as it leads to blood stream infections.[3] Tracheobronchial secretions in the intubated endotracheal tube acts as a sovereign risk factor for ventilator-associated pneumonia (VAP). These secretions promote *S. aureus* to form biofilm on the inner surface of endotracheal tube. Recent study on endotracheal tube mediated VAP has shown the prevalence of 22% of biofilm positive *S. aureus*.[4] *S. aureus* is also accounted for infective endocarditis in patients with cardiac prosthesis.[5] A multi-national study comprising 1779 patients with infective endocarditis has shown high prevalence of 31.4% of *S. aureus* in these infections.[6] Thus, the plethora of biofilm associated infections caused by *S. aureus* is unimaginable and surveillance studies to monitor the spread of *S. aureus* in hospitals are warranted.

Production of biofilm by *S. aureus* is primarily mediated by the intercellular adhesin operon (*ica* operon) which codes for the synthesis of polysaccharide intercellular adhesin (PIA).[7] Biofilm can also be triggered by intercellular adhesion independent factors such as biofilm associated protein (Bap), clumping factor A and B (ClfA and ClfB), Fibronectin binding proteins A and B (FnBpA and FnBpB), *Staphylococcus aureus* surface protein A (Spa), cidAB, *Staphylococcus aureus* surface proteins G and C.[8–10] So based on the presence or absence of intercellular adhesion genes, biofilm promoting genes in *S. aureus* are broadly classified into *ica*-dependent and *ica*-independent factors.

Apart from biofilm production, upsurge in *S. aureus* infections is mainly due to the acquisition of resistance to antibiotics belonging to beta-lactam group.[11] One such antibiotic is methicillin and the resistance to this antibiotic was reported within one year of its introduction. Methicillin Resistant *Staphylococcus aureus* (MRSA) has now emerged as a global pathogen in hospital- and community-associated infections. Like many other countries, even in India the prevalence of MRSA has been increased.[12] Resistance to methicillin in *S. aureus* is mainly mediated by the *mecA* gene carried on a mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*).[13] These are extremely diverse elements and based on the structural organization, they have been broadly classified into SCC*mec* type I to SCC*mec* XI.[14] Recent studies have shown that, in clinical isolates, the presence or absence of *mec*A gene influences the expression of biofilm phenotype.[15] Methicillin susceptible strains are known to show enhanced biofilm production by expressing PIA[16]; whereas, methicillin resistant strains formed biofilm in an *ica*-independent manner by secreting surface proteins or by releasing extracellular DNA.[17] These data indicates that there is an increasing need to study the occurrence of methicillin resistance in biofilm producing *S. aureus*.

MRSA are broadly classified into community associated-MRSA (CA-MRSA) and hospital associated-MRSA (HA-MRSA). A typical CA-MRSA strain will show heightened susceptibility to antimicrobial agents, except for antibiotics belonging to beta-lactam group. This suggests that most of the CA-MRSA strains carry SCC*mec* type IV and V as they consists of smaller genetic island when compared to SCC*mec* I–III which are mainly found in HA-MRSA strains.[18] Recent study from our lab has also shown the emergence of CA-MRSA of sequence type (ST) 2371 as a major clone among the clinical isolates collected from a tertiary care hospital in Mysuru, India.[19] These data clearly demonstrate the need for SCC*mec*-typing in device-associated *S. aureus*.

Presence of Panton-Valentine Leukocidin (*pvl*) gene is considered as a marker for CA-MRSA strains.[20] It is interesting to note that, *pvl* is less frequently reported in biofilm associated infections.[21] Hence, screening of *pvl* gene in device-associated isolates is necessary.

The present study was carried with an objective to identify the distribution of SCC*mec* types, *spa* types, *pvl*, antibiotic resistance, biofilm production and co-occurrence of *ica*-dependent and independent factors in device associated isolates. To the best of our knowledge, this is the first prevalence report on device-associated infections from Mysuru city, India which has a population of 3,001,127.[22]

Materials and methods

Sample collection

This is a cross-sectional study, wherein 200 ($n = 200$) clinical *S. aureus* strains isolated from infected implants over a period of one year (January 2014 to January 2015), were studied. These strains were obtained from the Department of Microbiology at Jagadguru Sri Shivarathreeswara (JSS) Medical Hospital and Krishnarajendra (KR) Hospital located in Mysuru, India. JSS medical hospital is a tertiary care centre with 1800 beds and it has 37 super-speciality

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