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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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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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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

Table 1
Types of patients, clinical conditions and samples used for the isolation of *S. aureus*.

Patient type	Implants used for patients' treatment	Clinical symptoms	Samples used for the isolation of *S. aureus*	Number of strains isolated ($n = 200$)
Patients with fracture of tibia, knee, intertrochanteric fracture, diaphyseal fracture of femur	Patients were treated with orthopaedic implants such as intramedullary nail, dynamic hip screw interlocking nail, stainless steel wire, locking plate	Fever, chills, redness around the knee joint, pus discharge and pain in the operated site	Swabs from orthopaedic implants and pus discharge	56 (28%)
Patients in intensive care units	Endotracheal tube	Fever, chills and septicemia within five days of hospitalization (early onset)	Tip of endotracheal tube	15 (7.5%)
		Fever, chills and septicemia after five days of hospitalization (late onset)	Tracheobronchial secretions	7 (3.5%)
Post-operative patients	Urinary catheters	High fever	Catheter tip	32 (16%)
Patients in intensive cardiac care units and on ventilators	Central venous catheters	Fever with septicaemia	Catheter tip, blood	32 (16%)
New born babies in neonatal intensive care units and patients with severe hypotension	Peripheral venous catheters	Fever with septicaemia	Catheter tip, blood	36 (18%)
Patients with open heart and by-pass surgery	Cardiac prosthesis	Fever with septicaemia	Swabs from mechanical heart valves, stents	22 (11%)

units. The KR hospital, another tertiary care hospital in Mysuru, has 1330 bed capacity and has more than 20 super-speciality units. Initial confirmation of *S. aureus* was done by the hospital staff using coagulase test, mannitol fermentation test and DNAse test. Later, molecular confirmation of *S. aureus* up to species level was done by PCR using the reported primers.[23] The American Association of Orthopaedic surgeons has classified orthopaedic prosthetic infections into four types: Type I – any two intraoperative cultures showing positive results, Type II – infection occurring within one month after surgery, Type III – infection in a working prosthesis caused by haematogenous seeding, Type IV – infection persisting in a patient for more than one month.[24] So based on the types, strains were isolated from orthopaedic implants. Types of patients, clinical conditions and the samples used for the isolation of *S. aureus* are shown in Table 1.

Screening for biofilm production

Biofilm screening by crystal violet method was performed using a modified protocol as described earlier.[25] Single colony of the isolate was inoculated to 25 ml trypticase soy broth with 0.5% glucose (TSB + 0.5% G) and incubated at 37 °C for 8–10 h until 0.5 OD is obtained. This culture was re-inoculated to fresh TSB + 0.5% G and incubated for 3–4 h to get 0.03 OD. 200 μl of this culture was inoculated to 96 wells flat bottom microtitre plate in triplicate and incubated at 37 °C for 48 h. *Staphylococcus aureus* ATCC 35556 was used as positive control and 3 wells with TSB + 0.5% G alone were kept as negative control. After incubation, plates were washed thrice with 0.15 M sterile PBS and stained with 0.1% crystal violet (HiMedia Labs, Mumbai, India) for 30 min at room temperature. Stained microtitre plates are washed thrice with sterile distilled water and dried in hot air oven at 45 °C. The bound dye was re-solubilised by adding 160 μl of 33% (v/v) glacial acetic acid to each well and kept for 30 minutes at room temperature. The re-solubilised dye was transferred to a new microtitre plate and OD was measured at 570 nm in a spectrophotometer (Thermo-Fischer Scientific). Based on the obtained OD values, quantification of biofilm strength was performed using the following formula.

At first the cut off OD (OD_c) was calculated using the formula

OD_c=Average OD of negative control + 3 × SD of negative control.

The OD_c was compared with the OD of samples (OD_s) and the strength of biofilm production observed in each isolate was assessed using the following formula

If $OD_s \leq OD_c$ then non-adherent

If $OD_c < OD_s \leq 2 \times OD_c$ then weakly adherent

If $2 \times OD_c < OD_s \leq 4 \times OD_c$ then moderately adherent

If $4 \times OD_c < OD_s < OD_c$ then strongly adherent

Antibiogram and MIC of biofilm positive isolates

Kirby-Bauer agar disc diffusion method was performed to determine the antibiogram of device associated strains.[26] Antibiotics used were cefoxitin, erythromycin, gentamicin, rifampicin, trimethoprim-sulphomethoxazole, tetracycline, clindamycin, linezolid and ciprofloxacin. The susceptibility to antibiotics was assessed as per the Clinical Laboratory Standard Institute (CLSI) guidelines.[27] MIC for oxacillin was calculated by agar-dilution method and the results were interpreted as per the CLSI guidelines.[27]

*SCC*mec*-typing*

MRSA isolates were screened for different *mec* types by multiplex PCR using the conditions as previously described.[28] Accuprime Taq DNA polymerase (Invitrogen, USA) was used for the multiplex-PCR. The control strains used for typing were *S. aureus* COL (type I), *S. aureus* BK2464 (type II), *S. aureus* ANS46 (type III), *S. aureus* MW2 (type IV), *S. aureus* WIS (type V) and *S. aureus* HDE288 (type VI).

Molecular screening of pvl, ica*-dependent and independent factors*

Molecular screening of ica-dependent, *ica*-independent and *pvl-luk f/s* genes were done by PCR. DNA was isolated using QIAamp DNA mini kit (Qiagen Cat. No. 51306) and the isolated DNA was used for PCR. The reaction was carried out in a 25 μl microfuge tube containing 2 μl of DNA, 11 μl of 2X PCR master mix (Cat. No. Beta Midsci™) and 12 μl of distilled water. Primers used for the detection of *S. aureus*, *pvl*, *ica*-dependent and independent factors

Table 2
Primer sequence, annealing temperature and amplicon size used for molecular characterization.

Primer name	Primer sequence	Annealing temperature	Amplicon size in bp	Reference
SA442 – F	AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG	55	108	23
SA442 – R	CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA			
icaAD – F	CCT AAC TAA CGA AAG GTA GG	55	1267	29
icaAD – R	TTA GCG TTG GGT ATT CCC TC			
clfA – F	ATT GGC GTG GCT TCA GTG CT	55	292	29
clfA – R	CGT TTC TTC CGT AGT TGC ATT TG			
clfB – F	ACA TCA GTA ATA GTA GGG GGC AAC	55	205	29
clfB – R	TTC GCA CTG TTT GTG TTT GCA C			
fnbpA – F	CAT AAA TTG GGA GCA GCA TCA	50	127	30
fnbpA – R	ATC AGC AGC TGA ATT CCC ATT			
fnbpB – F	GTA ACA GCT AAT GGT CGA ATT GAT ACT	55	524	30
fnbpB – R	CAA GTT CGA TAG GAG TAC TAT GTT C			
sasG – F	GTC AGT CAC TCA TAA CGC AGA G	50	400	This study
sasG – R	CCC AAG GAT TCC ACC ATT AGT T			
cidA – F	CCG GCA GTA TTG TTG GTC TAT T	50	280	This study
cidA – R	GCG TCT ACA CCT TTA CGA TGT T			
pvl – luk f/s – F	ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A	52	433	31
pvl – luk f/s – R	GCA TCA AGT GTA TTG GAT AGC AAA AGC			
spa-1113 – F	TAA AGA CGA TCC TTC GGT GAG C	59	Variable	32
spa-1514 – R	CAG CAG TAG TGC CGT TTG CTT			

are listed in Table 2 with the respective annealing temperature and amplicon size. PCR programme was set with an initial denaturation at 94 °C for 5 min followed by 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing and 60 s of extension at 72 °C. Final extension at 72 °C for 10 min was set as the last step of PCR programme. PCR was carried out in Eppendorf Master Cycler. 10 μl of amplified PCR products along with 1 kb DNA marker were loaded on 1.5% agarose gel and electrophoresis was performed. Amplicons were analyzed by using a gel documentation system.

spa *typing*

MRSA isolates were subjected to *Spa*-typing. The technique involves the sequencing of extremely variable X region in the staphylococcal protein A. Amplification of variable X region was performed using the primers described in Table 2. Amplified products were sequenced and the *spa* types were identified using Bionumerics version 6 software (Applied maths, Belgium).

MTT assay for the detection of antibiotic resistance in established biofilms

To check the effect of antibiotics on established biofilms, all the strong biofilm producing strains were subjected to MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium-bromide) assay. This was performed using the method described previously[33] with minor modifications. Based on the CLSI guidelines, resistant break point concentration (RBC) for linezolid, vancomycin and rifampicin was used in the MTT assay. The RBC for linezolid was set as 8 μg/ml, Vancomycin 16 μg/ml and rifampicin 4 μg/ml. Biofilms established on 24 well plates were treated with respective antibiotics for 1, 3 and 5 days. After the antibiotic treatment, wells were emptied and washed thrice with sterile PBS. Then, 100 μl of PBS with 1% MTT solution was added and incubated for 2 h at 37 °C. Later MTT was replaced by 100 μl dimethylsulphoxide (DMSO) and incubated for 15 min at room temperature. Bacteria with an active electron transport system reduced the tetrazolium salt to a water-soluble purple formazan product. This product was quantified by measuring the OD at 560 nm in a microplate reader. The inhibitory effect of antibiotics (OD_i) on established biofilms was studied by finding the ratio of OD values in presence of antibiotics and in the absence of antibiotics (control) on 1st, 3rd and the 5th day

$$OD_{inhibition}(OD_i) = \frac{OD_{560} \text{ in presence of antibiotics}}{OD_{560} \text{ in absence of antibiotics}}$$

The average OD_{560} (OD of 1st day, 3rd day and 5th day) of antibiotic treatment was compared with the OD_{560} values of respective controls of 1st day, 3rd day and 5th day. This represented the average inhibitory effect of a respective antibiotic $OD_{resistance}$ (OD_r). Decrease in OD_r value indicated greater biofilm inhibition by the respective antibiotic.

Results

Prevalence of MRSA, molecular confirmation of S. aureus *and screening of SCC*mec *types*

All the 200 device-associated isolates obtained from two hospitals were confirmed as *S. aureus* by PCR. High prevalence of SCC*mec* types IV and V was observed. Some of the strains were found to be non-typeable. The prevalence of MRSA and distribution of SCC*mec* types among device associated strains are shown in Table 3A.

Screening for biofilm production

Based on the crystal violet assay, high prevalence of strong biofilm producing strains was observed in orthopaedic implant associated isolates and the lowest was in the isolates from urinary catheters. Strength based characterization and number of strains isolated from infected devices are shown in Fig. 1.

Screening of pvl *and biofilm regulating genes*

Biofilm regulating genes, *icaAD*, were predominantly found in strong biofilm producing strains. Whereas, *ica*-independent genes were mainly found in moderate and weak biofilm producers. *icaAD* alone was found mainly in orthopaedic implants (28%). Interestingly, co-occurrence of *ica*-dependent and independent factors was higher in orthopaedic implants and peripheral venous catheters. Table 3B describes the occurrence of *pvl*, *ica*-dependent and independent genes in biofilm positive strains.

Antibiotic susceptibility test

Strains showing resistance to cefoxitin in disc diffusion method were identified as MRSA. These isolates were found to be

Table 3
Clinical and molecular characteristics of *S. aureus* strains isolated from infected medical implants.

A: Prevalence of MRSA isolates and SCC*mec* types

Infected implant	Infection type/ clinical symptoms	Number of strains isolated	Number of MRSA isolates	Isolates with MIC ≥ 256 μg/ml for oxacillin	*mecA*	SCC*mec* Type I	SCC*mec* Type II	SCC*mec* Type III	SCC*mec* Type IV	SCC*mec* Type V	SCC*mec* Type VI	Non typeable
Orthopaedic implants	Type I	19 (9.5%)	15 (7.5%)	15	15	4	2	1	5	3	0	0
	Type II	19 (9.5%)	12(6%)	12	12	2	1	2	6	1	0	0
	Type III	14 (7%)	14 (7%)	7	14	0	2	2	1	3	1	5
	Type IV	4 (2%)	1 (5%)	1	1	1	0	0	0	0	0	0
Endotracheal tube	Early onset	15 (7.5%)	5 (2.5%)	4	5	0	1	0	3	1	0	0
	Late onset	7 (3.5%)	4 (2%)	3	4	0	0	1	1	2	0	0
Urinary catheters	High Fever	32 (16%)	20 (10%)	17	20	4	1	1	3	2	0	9
Central venous catheters	Fever with septicaemia	32 (16%)	30 (15%)	18	30	2	1	2	11	5	1	8
Peripheral venous catheters	Fever with septicaemia	36 (18%)	25 (12.5%)	17	25	1	3	1	7	4	5	4
Cardiac prosthesis	Fever with septicaemia	22 (11%)	19 (9.5%)	11	19	3	2	5	4	3	1	1
Total		200 (*n* = 200)	145 (72.5%)	105 (52.5%)	145 (70%)	17 (8.5%)	13 (6.5%)	15 (7.5%)	41 (20.5%)	24 (12%)	8 (4%)	27 (13.5%)

B: Prevalence of biofilm regulating genes and PVL

Infected implant	Number of biofilm positive isolates	*icaAD*	*clfA*	*clfB*	*fnbpA*	*fnbpB*	*sasG*	*cidA*	*pvl* + SCC*mec* type IV	*pvl* + SCC*mec* type V	*pvl* + SCC*mec* type I–III
Orthopaedic implants	56 (28%)	24	11	13	4	10	22	14	15	17	3
Endotracheal tube	9 (4.5%)	4	2	7	5	3	2	0	4	2	0
Urinary catheters	5 (2.5%)	2	4	2	4	1	5	0	2	1	0
Central venous catheters	17 (8.5%)	7	2	5	10	3	3	2	5	6	1
Peripheral venous catheters	20 (10%)	15	6	3	7	4	10	4	7	1	1
Cardiac prosthesis	19 (9.5%)	13	3	7	9	8	5	3	5	7	0
Total	124 (62%)	65 (32.5%)	28 (14%)	37 (18.5%)	39 (19.5%)	29 (14.5%)	47 (23.5%)	23 (11.5%)	38 (19%)	34 (17%)	5 (2.5%)

C: Diversity of *Spa*-types among device associated MRSA strains

SCC*mec* types	Number of screened isolates	Frequently reported *spa* types	Number of frequently reported *spa* types
SCC*mec* type I	17	t307	15 (88.23%)
SCC*mec* type II	13	t018	11 (84.61%)
SCC*mec* type III	15	t363	14 (93.33%)
SCC*mec* type IV	41	t852	36 (87.8%)
SCC*mec* type V	24	t657	21 (87.5%)
SCC*mec* type VI	8	t172	4 (50%)

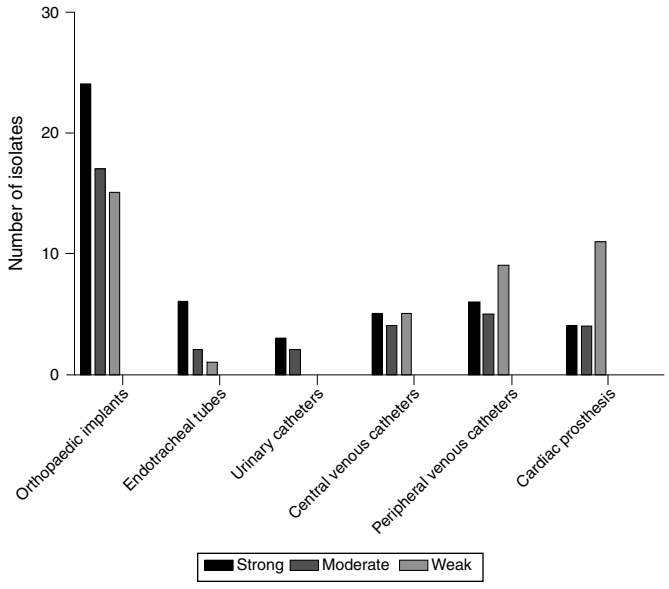

Fig. 1. Prevalence of biofilm positive strains and their respective strength.

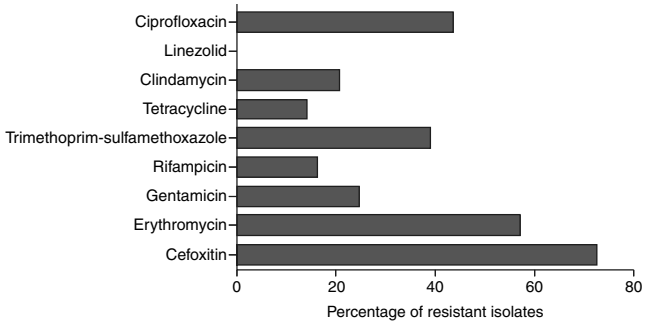

Fig. 2. Percentage resistance of antibiotics in device associated strains.

multi-drug resistant and the percentage of isolates resistant to each antibiotic is shown in Fig. 2.

spa *typing*

Overall 31 different *spa* types were identified among 200 device associated strains. *spa* type t852 and t657 were found to be higher among MRSA isolates. Strains with SCC*mec* type IV mainly belonged to *spa* type t852 and SCC*mec* type V belonged to t657. Table 3C

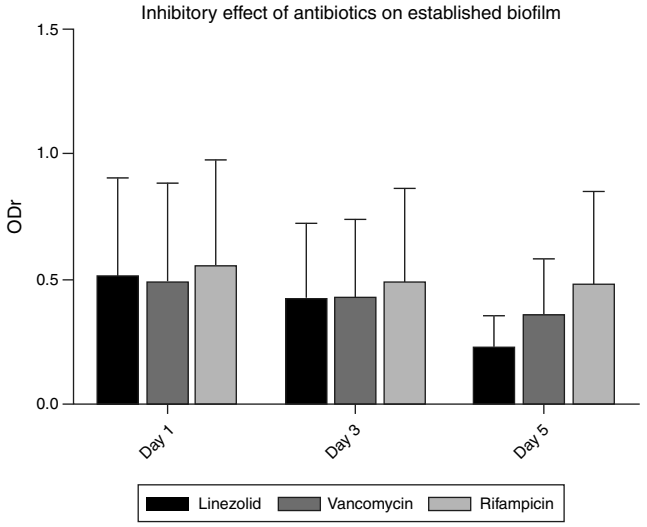

Fig. 3. MTT assay showing increased anti-biofilm effect by linezolid and vancomycin.

describes the distribution of *spa* types among the device associated strains.

Detection of antibiotic resistance in established biofilms

Vancomycin and linezolid at a concentration of 16 μg/ml and 8 μg/ml respectively, were effective in inhibiting established strong biofilms (Fig. 3). Among 48 strong biofilm producers, these two antibiotics controlled biofilm production in 33 strains. But in remaining 9 strains, the inhibitory effect of these antibiotics was less. These strains, possessed both *icaAD* (*ica*-dependent) and all the *ica*-independent factors (*fnbpA*, *fnBpB*, *cidA*, *clfA*, *clfB* and *sasG*).

Discussion

This is a first report on the prevalence and molecular profiling of device-associated *S. aureus* infections from Mysuru, India. Prevalence of device-associated infections was exceptionally high in isolates obtained from orthopaedic implants and peripheral venous catheters. In the present study, isolates from orthopaedic implants were able to form strong biofilms. This data suggests the increased risk of infections on orthopaedic implants through biofilm formation. Among the four types of orthopaedic implant associated infections, highest prevalence was observed in type I (9.5%) and type II (9.5%). The increased prevalence of orthopaedic device-associated infections, especially in type I and type II, warrants the need for proper hygiene practice during surgery.

Prevalence of peripheral venous catheter infections caused by *S. aureus* was moderate and the majority of strains showed weak biofilm phenotype. This could be due to their low duration of implantation. But some strains demonstrated strong biofilm phenotype, which foresee the essential role of *S. aureus* in PVC-associated infections. Device-associated infections were not limited to orthopaedic and peripheral venous catheters but were also observed in urinary catheters, central venous catheters and cardiac prosthesis. Their prevalence was comparatively low and they exhibited strong, moderate and weak biofilm phenotype.

Strains were further characterized for antibiotic susceptibility, SCC*mec* types, *pvl*, *spa*-types and biofilm regulating genes. The majority of isolated strains were resistant to methicillin (72.5%) and erythromycin (57%). Interestingly, resistance to gentamicin, clindamycin, rifampicin and tetracycline was mainly observed in the strains isolated from orthopaedic implants, cardiac prosthesis and peripheral venous catheters.

Minimal Inhibitory Concentration ≥256 μg/ml for oxacillin was observed in 105 (52.5%) strains. This high occurrence of extremely resistant MRSA strains in device-associated infections is alarming and thus screening for SCC*mec* types was performed. The recent trend in the epidemiology of CA-MRSA has shown the increased infiltration of these strains to hospitals.[34] In the present study, high prevalence of SCC*mec* type IV (20.5%) and V (12%) among device-associated strains was observed. This could be due to the possible persistence of CA-MRSA strains in these hospitals. Occurrence of *pvl* in clinical strains is a useful marker to identify CA-MRSA strains. Thus, *pvl* screening for all the MRSA isolates was performed and it was found in 38 SCC*mec* type IV and 34 SCC*mec* type V strains. This increased occurrence of *pvl* and SCC*mec* type IV and V, suggests the possible intrusion of CA-MRSA strains in hospitals.

The diversity of *S. aureus* strains in device-associated infections, was studied by *spa*-typing for MRSA isolates. *spa* types t852 and t657 were frequently observed in SCC*mec* types IV and V. Strains with SCC*mec* types I, II, III and VI mainly displayed t307, t018 and t363, t172 *Spa* types respectively. *spa* types t018, t852 and t657 are reported to belong to the clonal complex CC30, CC22 and CC1 respectively.[35] A recent study from Bangalore, south India, also showed the occurrence of t852, t3387 and t657 *spa* types in CA-MRSA strains.[36] This clearly indicates the distribution of these *spa* types in this geographical region.

Numerous predisposing factors have been identified in the formation of biofilm on device-associated infections. One such factor is the implant itself. But biofilm on an implant can also be interceded by the duration of implantation, patient's health condition, antibiotic therapy and the presence or absence of biofilm regulating genes. In this context, biofilm positive strains (MRSA and MSSA) were characterized for the presence or absence of intercellular adhesion-dependent and independent genes. High prevalence of *ica*AD was observed in device-associated strains (32.5%) mainly in orthopaedic implants, peripheral venous catheters and cardiac prosthesis. The presence of *mecA* gene and the occurrence of *icaAD* was compared in device associated isolates. Interestingly 20 MSSA strains (27.5%) possessed *icaAD* genes and revealed strong biofilm phenotype. Previous studies have established an important relationship between the expressions of *icaAD* in the absence of *mecA* gene.[16,17] Thus, the current findings are in concordance with previous reports and it signifies the essential role of MSSA strains in device associated infections. In contrast to this observation, a recent study has shown that the excision of *mecA* gene in MRSA strain BH1CC, reduced the expression of FnBp proteins.[18] This suggests the relation between *mec*A and FnBp proteins in biofilm expansion. In the present study an increased prevalence of *fnbpA* (19.5%) was identified in biofilm positive MRSA strains which substantiates the essential role of FnBpA in device-associated infections. Among the other *ica*-independent factors, the prevalence of *sasG* (23.5%) and *clfB* (18.5%) was high. Recent study has shown the mechanistic interaction of *S. aureus* SasG with *S. epidermidis* accumulation associated protein (Aap) through zinc ions.[37] Thus, the presence of SasG in device-associated strains may favour *S. epidermidis* infections through mixed-biofilm formation on medical implants.

MTT assay revealed the increased ability of some *S. aureus* strains to tolerate the effect of antibiotics. But the majority of strains were susceptible to linezolid and vancomycin; they showed their highest activity on the 5th day of treatment. Among the screened isolates, vancomycin, linezolid and rifampicin were could not remove biofilms in 9 isolates. Further analysis revealed that these isolates possessed *ica*-dependent and independent factors. This would have favoured their increased resistance to the tested antibiotics.

In conclusion, our study on device-associated infections in two major hospitals in Mysuru, showed a high rate of multidrug

resistant CA-MRSA strains. This underscores the need for routine surveillance of device-associated strains for implementing infection control strategies through proper antibiotic stewardship. Since medical implants promote biofilm formation, care must be taken while using these devices for patients' treatment. Our study warrants the need for effective guidelines to control *S. aureus* infections in patients with medical implants.

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Conflict of interest

The authors declare no conflict of interest.

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