Antibiotic susceptibility pattern of biofilm producing Staphylococci isolated from different clinical samples

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Keywords: biofilm, staphylococci, ica gene, antibiotic

Posted Date: March 31st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-19968/v1

Abstract

Background: Biofilm mediated infection by Staphylococci have a significant negative impact on patient health and necessitate reliable method for detecting biofilm producers. The ability of isolates to produce biofilm make them resistant to host immune response as well as available antibiotics. This study aims to detect biofilm producing ability among clinical staphylococci by phenotypic methods and presence of icaAD genes as well as their antibiotic profile.

Methods: A total of 4063 different clinical specimen received in the tertiary care hospital of Nepal were examined and Staphylococci were identified following standard microbiological procedure. The antibiotic resistivity pattern was detected by Kirby Bauer disc diffusion method whereas biofilm formation was detected by three phenotypic methods viz. congo red agar (CRA), tube method (TM) and tissue culture plate (TCP) method. Furthermore, icaAD genes were detected by PCR method.

Results: A total of 161 Staphylococci were isolated comprising S. aureus (63, 39.1%) and CNS (98, 60.9%). The isolates were found to be resistant to penicillin and erythromycin. Strong biofilm formation was detected among 6 (3.7%), 22 (13.7%) and 35 (21.7%) by CRA, TM and TCP method respectively. Similarly, among 24 (14.9%) isolates icaAD genes were detected. Biofilm formation was found to be correlated with methicillin resistance.

Conclusion: The study showed significant association between phenotypic production of biofilm and presence of ica genes. The biofilm producing isolates were found to be resistant to antibiotics than biofilm non producers.

Background

Staphylococci are Gram positive bacteria often residing as normal flora in human being but now emerging as one of the most common agent for hospital acquired infection [1]. Various diseases associated with this organism ranges from minor skin infection to life threatening endocarditis and septicemia. Staphylococci are most often associated with chronic infections of implanted medical devices [2, 3, 4]. Biofilm is a major virulent factor along with other agents that attribute to its pathogenesis. Biofilm infections characteristically are refractory to antibiotic treatments leading to treatment failures and relapse of infections. In addition, biofilms are also the source of metastatic infections because of their dispersal mechanism once they get matured [5]. According to the CDC, 65% of infections are associated with biofilms formed by the most notorious pathogens such as S. aureus, S. epidermidis and P. aeruginosa [6]. Biofilms can be defined as multicellular communities of bacteria, immobilized by an extracellular polymeric matrix produced by the bacteria, which can be attached to various biotic and abiotic surfaces [7]. The biofilm formation is mediated by Polysaccharide Intercellular Adhesion (PIA) which is 1, 6 linked 3 N-acetylglucosamine polymer responsible for cell-cell attachment and is the gene product of icaADBC [8]. Once grown as a biofilm, the embedded bacteria are protected from various physical, chemical and biological stresses. They develop high resistance to mechanical interference, mechanisms of innate and acquired host defenses, and antibiotic treatment. In fact, biofilms can resist antibiotic concentration 10–10,000 folds higher than those required to inhibit the growth of free floating bacteria [9, 10].

The knowledge on correlation of biofilm formation and antibiotic resistance as well as biofilm producing genes among clinical staphylococci will be helpful for preventive and therapeutic management of staphylococci infection and developing new strategies in their treatment.

Methods

The hospital based cross sectional descriptive study was conducted at KIST Medical College and hospital, Imadol, Lalitpur, Nepal. A total of 4063 different clinical samples like blood, urine, wound swab, different types of tips received in clinical microbiology lab of KIST Hospital were subjected to microbial analysis. All the clinical specimens were processed by standard microbiological technique as described by Cheesebrough [11]. The isolates were identified as staphylococci following Gram staining and different biochemical tests. Coagulase enzyme production by slide and tube method and DNase production were used to confirm the isolates as S. aureus. The species of CNS were identified based on simplified scheme proposed by Cunha et al. [12]. The antibiotic susceptibility test were performed towards various antibiotics by the modified Kirby Bauer disk diffusion method within the guidelines of Clinical and Laboratory Standard Institute (CLSI) [13].

Detection Of Biofilm Formation

Three phenotypic methods i.e. Congo Red Agar method (CRA), Tube method (TM) and Tissue Culture Plate Method (TCP) and polymerase chain reaction (PCR) for the detection of ica genes were used for detection of biofilm formation ability of isolates. All tests were performed using Staphylococcus epidermidis ATCC 35984 as positive control and repeated three times.

Congo red Agar method (CRA)

CRA medium was prepared with brain heart infusion broth 37 g/L, sucrose 50 g/L, agar (10 g/l) and Congo Red indicator 8 g/L. First Congo Red stain was prepared as a concentrated aqueous solution and autoclaved (121ºC for 15 minutes) separately from the other medium constituents. Then it was added to the autoclaved brain heart infusion agar with sucrose at 55ºC. CRA plates were inoculated with test organisms and incubated at 37ºC for 24 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production [14].

Tube Adherence method

A loopful of test organisms was inoculated in 10 mL of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37ºC for 24 h. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position and observed for biofilm formation. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilm formed was scored as 1 (weak/none), 2 (moderate) and 3 (high/strong) [15].

Tissue Culture Plate Method

Organisms isolated from fresh agar plates were inoculated in 10 mL of Brain Heart Infusion (BHI) broth supplemented with 2% sucrose. Broths were incubated at 37ºC for 24 h. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well flat bottom polystyrene tissue culture plates were filled

with 200 µL of the diluted cultures. The control organisms were also incubated, diluted and added to tissue culture plate. Negative control wells contained inoculated sterile broth. The plates were incubated at 37ºC for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times. This removed free floating bacteria. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA auto reader at wavelength 570 nm [15].

Table 1
Classification of Bacterial Adherence

Mean OD value	Adherence	Biofilm Formation
< 0.120	None	Non/weak
0.120–0.240	Moderate	Moderately Positive
> 0.240	Strong	Highly positive

Detection of ica genes

The genomic DNA was extracted as previously described using the DNA extraction Kit following the manufacturer instructions (Thermo Fischer).

The sequences of icaA and icaD (accession number U43366) were taken from the GenBank sequence of the National Center for Biotechnology Information (NCBI) database. Primers specific for icaA and icaD were designed by the Primer3 program and were purchased from Solis Biodyne (Denmark). The primer used for the detection of icaA was forward 5'-TCTCTTGCAGGAGCAATCAA and reverse 5'-TCAGGCACTAACATCCAGCA primer generating a product size of 188-bp. Similarly, for detection of icaD, 5'-ATGGTCAAGCCCAGACAGAG was used as a forward primer and 5'-CGTGTTTTCAACATTTAATGCAA was used as a reverse primer with the product size of 198 bp. The PCR product was analyzed in 2% agarose gel stained with SYBR safe (Invitrogen) dye.

Data analysis

The statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, United States) software. Chi-square test was used to compare between groups of clinical isolates and P-values < 0.05 were considered statistically significant.

Results

Among 4063 samples analyzed, 654 showed significant growth where 161 were identified as Staphylococci. Five species were identified among all CNS isolates including S. epidermidis (59.2%); the most frequently isolated species followed by S. saprophyticus (19.4%), S. haemolyticus (9.2%), S. homonis (8.2%) and S. capitis (4.1%). Among 161 Staphylococcal isolates, S. aureus were isolated in high number from W/P (47, 29.2%) whereas CNS were isolated from blood (54, 33.5%) (Table 2).

Table 2

Distribution of staphylococcal isolates among different clinical sample

Isolates	Clinical sample					
	CVC	Blood	w/p	Urine	Tips	Total
S. aureus	5 (3.1%)	6 (3.7%)	47 (29.2%)	-	5 (3.1%)	63 (39.1%)
CNS	14 (8.7%)	54 (33.5%)	7 (4.3%)	11 (6.8%)	12 (7.5%)	98 (60.9%)

CVC = central venous catheter, w/p = wound/pus, tips = catheter tips, suction tips, drain tips, DJ stenting tips, transtracheal tips

Antibiotic susceptibility profile of isolates

S. aureus were found to be sensitive towards commonly used antibiotics as tetracycline (100%), chloramphenicol (98.4%) and clindamycin (87.3%) but found to be resistant towards penicillin (95.2%) and erythromycin (93.6%). Similarly, CNS were also found to be resistant towards penicillin (93.9%) and erythromycin (75.5%) and sensitive towards chloramphenicol (92.9%), tetracycline (86.7%) and clindamycin (72.4%) As indicated by cefoxitin disc diffusion assay, 56 (89%) of S. aureus were methicillin resistant and 65 (66%) were methicillin resistant CNS (Table 3).

Table 3 Antibiotic resistant pattern of Staphylococci

Antibitoics	Potency (µg/disc)	Resistant cases		Total (n=161)
		S. aureus (n=63)	CNS (n=98)	
Penicillin	10 units	60 (95.2%)	92 (93.9%)	152 (94.4%)
Ciprofloxacin	5	41 (65.1%)	31 (31.6%)	72 (44.7%)
Tetracycline	30	-	13 (13.3%)	13 (8.1%)
Clindamycin	2	8 (12.7%)	27 (27.5%)	35 (21.7%)
Chloramphenicol	30	1 (1.6%)	7 (7.1%)	8 (5%)
Cefoxitin	30	56 (88.9%)	65 (66.3%)	121 (75.2%)
Erythromycin	15	59 (93.6%)	74 (75.5%)	133 (82.6%)
Cotrimoxazole	1.25/23.75	34 (54.0%)	37 (37.7%)	71 (44.1%)
Gentamycin	10	14 (22.2%)	13 (13.3%)	27 (16.8%)

Detection Of Biofilm Formation

Among all the Staphylococci isolates, black colonies were produced by 6 (3.7%) isolates in CRA while 16 (10%) isolates were moderate biofilm producers that showed Bordeaux colored colonies. Remaining 139 (86.3%)

isolates were found to be biofilm non- producers whose colony color was pink to red. Strong biofilm production was observed only among CNS.

By TM method, the biofilm production was observed among 6 (3.7%) S. aureus and 9 (5.6%) CNS. While TCP method detected 5(3.1%) biofilm producers among S. aureus and 14 (8.7%) among CNS. In total of 161 isolates, 24 (14.9%) isolates were found to possess both icaA and icaD genes comprising 6 (3.7%) S. aureus and 18 (11.2%) CNS isolates. None of the genes were identified in 137 (85.1%) isolates. (Table 4).

Table 4
Detection of biofilm formation among Staphylococci by different phenotypic and genotypic methods

Method	Biofilm formation	No. of isolates (n)		Total (n = 161)
		S. aureus (n = 63)	CNS (n = 98)	
CRA method	Strong	-	6 (6.1%)	6 (3.7%)
	Moderate	1 (1.6%)	15 (15.3%)	16 (9.9%)
	Weak/Non	62 (98.4%)	77 (78.6%)	139 (86.3%)
TM method	Strong	3 (4.8%)	19 (19.4%)	22 (13.7%)
	Moderate	8 (12.7%)	8 (8.2%)	16 (9.9%)
	Weak/Non	52 (82.5%)	71 (72.4%)	123 (76.4%)
TCP method	Strong	21 (33.3%)	14 (14.3%)	35 (21.7%)
	Moderate	14 (22.2%)	28 (28.6%)	42 (26.1%)
	Weak/Non	28 (44.4%)	56 (57.1%)	84 (52.2%)
Detection of ica gene	Presence	6 (9.5%)	18 (18.4%)	24 (14.9%)
	Absence	57 (90.5%)	80 (81.6%)	137 (85.1%)

Methicillin resistivity among ica positive isolates

In total of 161 isolates, 24 (14.9%) isolates were found to possess both icaA and icaD genes comprising 6 (3.7%) S. aureus and 18 (11.2%) CNS isolates. None of the genes were identified in 137 (85.1%) isolates. The ica genes were harbored by methicillin resistant than methicillin sensitive isolates of both S. aureus and CNS (Table 5).

Table 5
Presence of ica gene among Staphylococci

ica genes	MRSA	MSSA	P value	MRCNS	MSCNS	P value	Total
Presence	4 (6.3%)	2 (3.2%)	0.069	14 (14.3%)	4 (4.1%)	0.255	24 (14.9%)
Absence	52 (82.5%)	5 (7.9%)		51 (52.0%)	29 (29.6%)		137 (85.1%)

Evaluation of different methods for the detection of biofilm production

When different methods for the detection of biofilm formation was analyzed, it was found that TM method is statistically significant when compared with presence of ica genes whereas other two phenotypic methods were statistically insignificant (Table 6).

Table 6
Screening of biofilm formation by different methods

Biofilm formation	CRA	TM	TCP	ica genes
High	6 (3.7%)	22 (13.7%)	35 (21.7%)	24 (14.9%)
Moderate	16 (10.0%)	16 (9.9%)	42 (26.1%)	
Weak/non	139 (86.3%)	123 (76.4%)	84 (52.2%)	137 (85.1%)
P value	0.268	0.000	0.272	

Antibiotic Resistant Pattern Among Biofilm Positive Isolates

The biofilm positive isolates as detected by TM and TCP method were found to be resistant to penicillin (90% & 94%) and erythromycin (71% & 82%) respectively. Similarly, those isolates which possess icaAD genes were resistant to penicillin (100%) and erythromycin (83%) (Table 7).

Table 7
Antibiotic resistivity and biofilm formation by different methods

Antibiotics	Biofilm detection methods		
	TM method (n = 38)	TCP method (n = 77)	icaAD genes (n = 24)
Penicillin	34 (89.5%)	72 (93.5%)	24 (100%)
Ciprofloxacin	20 (52.6%)	36(46.8%)	10(41.7%)
Tetracycline	4 (10.5%)	4 (5.2%)	1 (4.2%)
Clindamycin	8 (21.1%)	15 (19.5%)	5 (20.8%)
Chloramphenicol	1 (2.6%)	3 (3.9%)	1 (4.2%)
Cefoxitin	25 (65.8%)	58(75.3%)	18(75%)
Erythromycin	27(71.1%)	63(81.8%)	20 (83.3%)
Cotrimoxazole	12 (31.6%)	36(46.8%)	10 (41.7%)
Gentamycin	3 (7.9%)	10 (13%)	1 (4.2%)

Discussion

Even though, most Staphylococci are present as normal flora in human body, they remain a versatile and potent pathogen since it is one of the most common cause of nosocomial and community acquired infection. They are associated significantly with various self-limiting to severe life threatening infection due to its ability to produce biofilm on inert as well as living tissues [2].

A total of 161 clinically significant Staphylococci were studied. More than half of the isolates were CNS (98, 61%) as compared to S. aureus (63, 39%). Some studies have found high number of S. aureus than CNS [16–18]. The study by Bose et al. shows 111 (62.01%) were S. epidermidis and 68 (37.99%) were S. aureus among 179 Staphylococcal isolates [19]. In a study carried out by Gad et al. [20], out of 292 isolates of urine and catheter, 53 (18.2%) staphylococcal strains were identified (S. aureus represented 6.2% and S. epidermidis represented 12%).

Among the isolates, five different species of coagulase-negative staphylococci were encountered; S. epidermidis (58, 59%), S. saprophyticus (19, 19%), S. haemolyticus (9, 9%), S. hominis (8, 8%) and S. capitis (4, 4%). The present results reveal that S. epidermidis are the most frequently isolated species. The findings of the present study are in agreement with the various studies which shows S. epidermidis as the most common CNS [21–23]. Staphylococci are commensal of skin and commonly gain access to site of skin puncture and deep cuts which most time cause uncomplicated infections but at times may develop into complicated infections leading to systemic failure [24]. It has been noticed in several studies that the S. epidermidis is the most frequently isolated species in nosocomial infections.

Staphylococci are commensals as well as pathogens of human beings and because of their versatile nature they were isolated from different clinical samples. Out of 161 Staphylococci, the highest number of CNS were isolated from blood 54 (33.5%) and S. aureus from W/P 47(29.2%). Increased antibiotic resistance, in addition to the increased frequency of invasive surgery, increased use of intra vascular devices, and increased number of patients with immune compromised status because of HIV infection or immunosuppression after transplantation or cancer treatment, has led to sharp increases in the incidence of S. aureus bacteremia and S. aureus infective endocarditis [25, 26] and is associated with significant mortality and morbidity. Bloodstream infection with the S. aureus is associated with mortality rate of about 30% and the incidence is increasing [27].

In order to fight bacterial infections successfully, the rapid recognition of proper treatment modalities are critical. The determination of antibiotic susceptibility and resistance are keys to this process [28]. Resistance has been observed to every class of antibiotic, regardless of whether it was derived from natural or synthetic origins. The emergence of antimicrobial resistance among Staphylococci isolates is one of the important factor in nosocomial infection. About 90% of the S. aureus strains found in hospitals are now resistance with penicillin G. With the extensive exploitation of therapeutic agents, CNS also have lost its susceptibility to most of the available antibiotics and become resistance to most active antimicrobials that is β lactams and other antimicrobial classes [29]. Both S. aureus and CNS were found to be resistant to penicillin 60 (95.2%) and 92 (93.9%) followed by erythromycin 59 (93.6%) and 74 (75.5%) respectively. Fortunately, the S. aureus and CNS were found to be susceptible to common antibiotics as tetracycline (100%), 85 (86.7%) and chloramphenicol 62 (98.4%) and 91 (92.9%) respectively.

S. aureus infections are very common and MRSA continues to be a serious and dreadful challenge as their prevalence is reported to be increasing exponentially. Due to the extensive exploitation of therapeutic agents,

CNS also have lost susceptibility to many antibiotics and generating a major problem [30]. The present study reported MRSA as 56(34.8%), MSSA as 7(4.3%), MRCNS as 65(40.4%) and MSCNS as 33(20.5%) among 161 Staphylococci. The prevalence of MRSA is 47.05% (48) among 102 S. aureus which is lower than the result reported from south India [31]. In studies carried out in similar settings in Nepal, 75.6%, 69.1% and 54.9% MRSA were reported, fairly higher than present study [32–34]. The difference in prevalence of MRSA may be because of the factors like healthcare facilities available in the particular hospital and rationale antibiotics usage which varies among hospital in different parts of the world. The important reservoirs of MRSA in hospitals/ institutions are infected or colonized patients and transient hand carriage is the predominant mode for patient to patient transmission. But the considerable increase in the prevalence of MRSA has been observed globally [31]. Likewise prevalence of MRCNS is (12) 25% among 48 CNS isolates which was in accordance with other studies [27, 35, 36] but opposed with the findings of others [26]. Similarly, prevalence of MRCNS ranging from 48.2–60% has been reported in India [6] which was comparatively higher than our study. Overall, data indicated by this study shows slightly lower rate of MRSA and MRCNS than that reported by other studies.

Pathogenesis of Staphylococci is attributed to a number of virulence factor and biofilm formation is thought to be the most important one. There are number of methods available for biofilm detection. Both phenotypic and genotypic methods were used to analyze the ability of biofilm production in all isolates. Growth of organism on the surface of CRA media is simple, easy and inexpensive method for detection of slime production. Investigation of biofilm by CRA showed 22 (13.7%) staphylococcal isolates positive for the slime production. Among CRA positive, only 6 isolates formed black colonies representing the strong biofilm production. Variable result was obtained from various researches [19, 21, 23, 37]. Slime formation is not always indicative of biofilm formation in vivo as highlighted by Arciola et al. [38} and Mathur et al. [39]. The consistency and color of the colony developed depends not only strains of bacteria, nutrient composition, origin of specimen, physiology of isolates as well as incubation time.

Investigation of biofilm production by the tube method showed 24 (14.9%) isolates as strong biofilm producers, 16 (9.9%) moderate and 121 (75.2%) weak/non-biofilm producers. This result is comparable with Mathur et al. [39] (11.8%) but the data is less than that observed by other researchers [40, 41]. The result of tube method is based on visual observation of adherent on the wall of tube. So, it is difficult to discriminate between weak and biofilm negative isolates due to the variability in observed result by different observers.

The TCP method detected 35 (21.7%) strong and 84 (52.2%) weak biofilm producers. The TCP method is a convenient and quantitative technique that directly detects the polysaccharide production by measuring the adherent biofilm by spectrophotometer. TCP is the most widely used and was considered as standard test for the detection of biofilm formation [20, 39]. This method has been reported to be the most sensitive, accurate and reproducible screening method for the determination of biofilm production by clinical isolates of Staphylococci and has the advantage of being a quantitative tool for comparing the adherence of different strains [15, 39].

Previous studies have shown the presence of ica locus in clinical isolates emphasizing their increased virulence as compared to the saprophytic strains [44, 42]. Besides, plethora of studies has demonstrated the causal link between staphylococcal biofilm and the presence of ica operon (icaADBC genes) [38, 43, 44], which in turn are involved in the PIA production; the most extensively characterized staphylococcal biofilm component. In ica operon, mainly co-expression of icaA and icaD has been demonstrated to be necessary for phenotypic

expression of biofilm production in clinical staphylococcal isolates [23, 43]. Besides, being reliable yet efficient, PCR of ica genes has been extensively used for the detection of biofilm formation [20, 23, 43]. In the present study, concomitant presence of icaA and icaD genes was detected in 24 (14.9%) staphylococcal isolates comprising of 6 (3.7%) S. aureus and 18 (11.2%) CNS isolates. Previous studies have also demonstrated the presence of ica genes in clinical staphylococcal isolates. Los et al. [45] showed the prevalence of ica operon in 27.4% nasopharyngeal S. epidermidis isolates from hospitalized patients. Oliveira & Cunha [23] detected ica genes in 40% CNS isolated from clinical specimen and nares of healthy individuals. Likewise, Cafiso et al. [46] found 35% of the isolates positive for icaA and icaD genes, Silva et al. [47] showed 40% staphylococcal isolates positive for ica genes respectively. Altogether, these results indicate importance of ica genes in biofilm production in device associated infections.

This low rate of ica detection as compared to the previous studies [21, 23, 38, 45, 46] may be due to difference in in-vivo and in-vitro conditions possibly contributing to the physiological changes of the pathogen modulating biofilm formation capabilities. For instance, ica genes are expressed in the stressful environment such as high osmolarity, anaerobic condition, high temperature, and sub-inhibitory presence of some antibiotics [17, 38]. Studies have demonstrated biofilm formation via PIA-independent mechanisms in S. aureus [48]. A number of transcriptional regulators have been reported in ica-independent biofilm production. These include araC- type transcriptional regulator or regulator of biofilm (rbf), which controls the biofilm production by novel regulatory mechanism [49]. Likewise, biofilm-associated protein (Bap); the first gene known to form biofilm via icaADBC independent in S. aureus from bovine mastitis isolates. Although initially, it appeared to be absent in human clinical S. aureus isolates, Bap protein has now emerged associated with more than 100 surface proteins that are involved in biofilm formation [50]. In the clinical S. aureus isolates of UAMS-1 strain, mutation of ica locus showed little effect on biofilm formation, thus, suggesting the presence of additional loci relevant to biofilm formation [24]. Also, studies suggest the regulation of biofilm by global regulator SarA in ica-independent mechanisms [43]. However, given the undeniable role of icaADBC in biofilm matrix formation and that PCR enables rapid diagnosis of slime producing virulent strains assays; implementation of genotypic measure is strongly suggested in routine diagnostic laboratory. We reason many factors as environment, nutrition, sub inhibitory concentration of certain antibiotics, and stress (temperature, osmolarity) might play a significant role in biofilm formation resulting in varied frequency of biofilm producers among clinical isolates [17, 38, 48].

From a clinical perspective, the discrepancy between genotype and phenotypic resistance expression suggest that a susceptible strain harboring, but not expressing, an antibiotic resistance gene should be regarded as potentially resistant to that antibiotic. Overall, we did not detect a significant presence of antibiotic resistance genes, compared to the great biofilm resistance of the isolates [45].

In consistence with previous studies, CRA and TCP method correlated well in positive results [23, 39, 46]. However, evidences of false negative results in CRA method while comparing with TCP method suggest that CRA method alone cannot be solely depended upon for the precise detection of biofilm formation. Taken together, in this study, the modified TM method showed the best correlated result with genotypic assay suggesting its importance in routine diagnostic laboratories. Oliveira & Cunha [23] also reported good sensitivity and specificity for the tube test and PCR when analyzing isolates obtained from infection. According to Cunha et al. [12], the test provides reliable results for biofilm detection in CNS and is adequate for routine use.

Conclusion

The study showed a significant association in between phenotypic production of biofilm and presence of ica genes. Taken together, this study demonstrates the high prevalence of methicillin resistant isolates producing biofilms in clinical staphylococcal samples. Since staphylococcal infections have a significant impact on morbidity and mortality, prevention and management of these infections remain a priority. This study, while bringing additional information about the status of biofilm producing clinical strains and their association with multiple antibiotic resistances, highlights the importance of early detection strategies in routine diagnostics. Implementation of those will help to identify biofilm producing cases to prevent occurrence of treatment failures of staphylococcal infections in Nepal.

Limitation Of The Study

The study of all genes responsible for biofilm production other than icaA and icaD genes could not be carried out. The presence of ica genes were not tested with antibiotic resistant genes to confirm the resistivity.

Abbreviations

CRA
Congo Red Agar
TM
Tube method
TCP
Tissue Culture Plate
CNS
Coagulase negative Staphylococci
CLSI
Clinical and Laboratory Standards Institute
BHI
Brain Heart Infusion

Declarations

Acknowledgements

Not applicable

Authors' contributions

SM, MC conceptualized and designed the study. RS processed, analyzed and carried out the experiment. RS and SL analyzed the result and drafted the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by small RDI grant from UGC, Nepal (SRDIG/72-73/HS-15).

Availability of data and materials

All the dataset of this article is available from the corresponding author if reasonably requested.

Ethics approval and consent to participate

The ethical clearance and consent to participate was approved by Nepal Health Research Council (Reg. no. 213/2015) and Institutional Review Committee (IRC) of KIST hospital.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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