thod, with the presence of *Ica AD* genes, 46% (24/52) isolates were non-biofilm forming when assessed by MTP and 39% (20/52) isolates were observed as non-biofilm formers by CRA method but were positive for *Ica A* or *Ica D* genes. Similarly, 31% (16/52) and 13% (9/52) isolates were non-biofilm formers by MTP and CRA method, respectively, but were positive for *Ica B* or *Ica C* genes. About 15% (8/52) and 25% (13/52) of isolates were biofilm formers by MTP method and CRA, respectively, despite the absence of *Ica AD* genes. Similarly, 33% (17/52) and 52% (27/52) of isolates were observed as biofilm formers on MTP and CRA but were negative for *Ica BC* genes. But only 6% (3/52) of biofilm formers by MTP and CRA method were positive for whole *Ica* operon while 10% (5/52) and 8% (4/52) non-biofilm isolates by MTP and CRA method were observed as positive for complete *Ica* operon.

Association of antibiotic resistance with biofilm forming ability of Staphylococci

Biofilm former strains showed higher resistance than the non-former isolates except for tobramycin, amikacin and ciprofloxacin. Highest resistance among the biofilm formers was observed for cephalothin, ceftriaxone, cefoxitin, penicillin, tetracycline and chloramphenicol from 33.33-82% (Table 3).

Table 3. Antimicrobial resistance pattern exhibited by biofilm former and non-biofilm former oral *Staphylococci*

Antibiotic		**Biofilm formers** *n* **(%)**	**Non-biofilm formers** *n* **(%)**
Aminoglycosides	TOB 30µg	06 (18.18)	04 (21.0)
	AK 30µg	01 (3.03)	01 (5.26)
Fluoroquinolones	CIP 5µg	05 (15.15)	05 (26.31)
	OFX 5µg	07 (21.21)	02 (11.0)
Cephalosporins	KF 30µg	27 (82.0)	12 (63.0)
	CRO 30µg	19 (58.0)	06 (32.0)
Sulfonamides	TMP/SMX µg	07 (21.21)	03 (16.0)
Cefoxitin	FOX µg	19 (58.0)	06 (32.0)
Tetracycline	TE 30µg	11 (33.33)	05 (26.31)
Penicillin	AML 10µg	17 (52.0)	06(32.0)
Lincosamide	DA 10µg	03 (9.09)	01 (5.26)
Nitrofurantoin	F 300µg	07 (21.21)	01 (5.26)
Chloramphenicol	C 30µg	11 (33.33)	04 (21.0)
Linezolid	LZD 30µg	07(21.21)	01 (5.26)

Comparative analysis of detection methods used for biofilm formation

Sensitivity and specificity of PCR was estimated to be 100% and 86% respectively, with 94% accuracy. CRA and MTP had a relatively acceptable sensitivity (69% and 65%) and low specificity (45% and 38% respectively) to identify biofilm phenotype, when compared to PCR (Table 4).

Table 4. Comparison of different diagnostic parameters for testing validity of CRA, MTP and PCR methods for *Staphylococci* biofilm detection

Statistical	**SN**	**SP**	**PPV**	**NPV**	**PLR**	**NLR**	**Accuracy**

parameters	(%)	(%)	(%)	(%)	(%)	(%)	(%)
CRA	69	45	67	47	1.56	1.51	60
MTP	65	38	40	63	1.75	1.68	48
PCR	100	86	91	100	1.17	1.15	94

SN; Sensitivity, **SP**; Specificity, **PPV**; Positive Predictive Value, **NPV**; Negative Predictive Value, **PLR**; Positive Likelihood Ratio, **NLR**; Negative Likelihood Ratio.

Discussion

In present study, evaluation of the isolation frequency of *S. aureus* and *S. epidermidis* from oral cavity of healthy adults along with its antibiotic susceptibility profile and biofilm forming ability was determined. Furthermore, comparative analysis of methods which were used for the detection of biofilm formation by antibiotic resistant oral *Staphylococci* was also carried out**.** It was found from present work that *S. aureus* (36/52, 69.2%) was the commonly isolated organism from saliva samples followed by *S. epidermidis* (16/52, 30.7%). Antibiotic susceptibility testing of these isolates showed that majority of isolates were sensitive to the commonly used antibiotics. Resistance was seen against cephalosporin of first and third generation i.e. 57% against cephalothin and 52% against ceftriaxone. Results from the present study were in line with the study conducted by Bello *et al*., (2013) and Rahman *et al*., (2015) which reported that majority of oral bacteria showed susceptibility to most of the antibiotics and no single bacteria was resistant to amikacin, amoxicillin and imipenem. Contrary results were reported by Silva *et al*., (2016), where amoxicillin was an ineffective drugs because of its high resistance while in current work majority of isolates were susceptible to amoxicillin. The methicillin resistance rate for *S. aureus* observed in this study was 31% which was similar to the results of Bueris *et al*., (2005). Although most of the oral bacteria from present study showed antibiotic sensitivity, even low resistance can be a serious health risk and lead to the spread of antibiotic resistance. Furthermore, the detection of MRSA strains indicates that they are already defunded at the community, and thus these individuals may serve as a reservoir and source of multi-resistant pathogenic *S*. *aureus* strains.

In present work, results showed that 38% and 62% of *Staphylococcus* isolates exhibit biofilm production by MTP Rehman et awhere only 23% of isolates had a biofilm phenotype identified by both CRA and MTP. Kord et al., (2018) also reported no correlation between CRA and MTP method as only 9.7% of isolates were identified as biofilm formers by both methods. In our study, 38% isolates were biofilm formers by MTP method which was very close to the findings of Arciola *et al*., (2001) and Liduma *et al*., (2012). However, similar to our study, lower rates of biofilm phenotype by MTP was reported by Gad *et al*., (2009) and Saising *et al*., (2012) from Egyptian and Thailand population respectively. These discrepancies in results could be due to the use of different spectrophotometric procedures and sources of clinical specimen (Abou El-Khier et al., 2015), and also attributed to the fact that phenotypic expression of biofilm formation is highly sensitive to *in vitro* conditions and hence can be detected variably by different methods (Shrestha *et al*., 2018). CRA method results from the present study were in complete disagreement with the study carried by Kord *et al*., (2018) and Hassan et al., (2011). Similar to the present work, results reported by El-Mahallawy *et al*., (2009) and Abou El-Khier et al., (2015). PCR is used as a gold standard method for biofilm detection (Rampelotto *et al*., 2018). PCR amplification of *Ica* gene locus represents that *Ica A* and *Ica D* were most detected (77% and 57% respectively) while, *Ica B* and *Ica C* genes were least prevalent genes (38% and 27%, respectively) expressed by *Staphylococcus* isolates. Current findings were in accordance with the reports of Gad *et al*., (2009). There were some variants in present study which were non-biofilm formers by phenotypic methods but were *Ica* positive. Chromosomal point mutations, post-translational regulation and negative translational mechanisms may be the reasons of non-biofilm formation in strains in which *Ica* locus is present and thus affects the production of biofilm associated proteins (Abu Taleb *et al*., 2012; Darwish & Asfour, 2013; Los *et al*., 2010). Some isolates were observed as biofilm formers by phenotypic methods but were

detected negative for *Ica* genes suggesting that this may be due to an *Ica* gene-independent control of biofilm formation/adhesion process in *Staphylococci* (Kord *et al*., 2018). Biofilm forming bacteria are usually less susceptible to antibiotics than planktonic bacteria (Pinheiro *et al*., 2014; Shrestha *et al*., 2018). Biofilm former strains from the present work showed higher resistance than the non-former isolates except for tobramycin, amikacin, and ciprofloxacin. Our findings are somewhat similar to the work conducted by Shrestha *et al*., (2018).

Conclusion

The current study showed that PCR method is more appropriate, as it is less costly as well as less likely that results would be misinterpreted. In contrast, CRA, although easier and faster to perform but less sensitive and therefore, cannot be recommended as a screening test for identifying biofilm production by *Staphylococcus* species. Biofilm forming ability also enhanced antibiotic resistance. Moreover, the present study revealed that the presence of *Ica* genes alone does not lead to biofilm formation. On the other hand, the biofilm-forming ability of some isolates in the absence of *Ica* genes emphasizes the importance of *Ica*-independent mechanisms of biofilm formation.

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