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ORIGINAL ARTICLE

MSCRAMMs Encoding Genes Expression in MRSA Isolated from Children with Cleft Lip and Palate and its Relation to the Phenotypic Ability of Biofilm Formation

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ABSTRACT

Objectives: *Our study aimed to detect the correlation between the MSCRAMMs gene expression and the ability of MRSA to produce biofilm phenotypically.* ***Methodology:*** *We studied staph aureus isolated from cleft lip and cleft palate of congenital mal or deformed children. Total of 100 isolates were collected from cleft lip and palate of children from plastic surgery department of Tanta University Hospital during a period of 11 months. All samples were subjected to phenotypic methods then biofilm forming strains were assessed for genes by multiplex PCR.* ***Results:*** *85/100 of samples were stap. Aureus 28/85 were MRSA .Phenotypic aspect showed that 56% of strains were high biofilm producers while 30% were intermediate and 14% were low producer. fib gene was the most prevalent gene among tested strains producing biofilm.* ***Conclusion****: MRSA harbor MSCRAMMs encoding gene family, There are several genes icaA&B&D & eno &ebps anf fib are the targeted in our study. There is a strong correlation between ability of biofilm formation and its genotypic control.*

INTRODUCTION

Microorganisms growing in a biofilm are associated with chronic and recurrent human infections and are highly resistant to antimicrobial agents. *Staph. aureus* can cause a variety of infections with a special interest to strains harboring genes encoding for biofilm formation.[1] Biofilm formation in MRSA Is under the control of variety of intracellular adhesion molecules that are controlled by specific genes. Various strains of staph aureus vary in the pattern of prevalence of these genes. These genes encoding for proteins and these proteins include: clumping factor A&B (ClfA & ClfB) &- fnbA & B (Fibronectin binding proteins A&B).[2-3]

Eno(Laminin binding protein) & Fib (Fibrinogen binding protein)& Can(Collagen binding protein). Children with unrepaired cleft lip or palate can significantly harbor and carry MRSA than normal well-formed lips and palate having children.[4-6]

Various *staph aureus* may not have the same prevalence of MSCRAMMs encoding genes and this can make different predisposition of individual to a variety of infections. [7-8]

METHODOLOGY

A total of 100 child with unrepaired cleft lip or palate were selected randomly from Plastic surgery Department of Tanta University Hospital during the period from January 2018 to December 2018 over a period of 11 months. The study was done after the approval of ethical committee in Tanta Faculty of Medicine and a written consent from the parents of the participated patients. Twenty normal healthy child were used as a control group. The control group included normal children without any deformities in lips or palates and were in the same age group of patient group.

All patients were under age of 6 years and all patients were immunocopetent. All patients and control groups were swabbed and swabs were transmitted immediately to the Microbiology Laboratory. Swabs were immersed in nutrient broth and the tubes were incubated for 24 hours at 37°C

Subculture on nutrient agar then incubation at 37°C for 24 hours was done. Identification of *staph aureus* by colonial morphology & stained film with gram stain & mannitol fementation& mannitol salt agar and coagulase test were done.

Detection of MRSA

MRSA was detected by oxacillin disc (10ug) during antibiogram using other discs:Targocid (30ug) ,Ciprofloxacin (5ug), LZN(30ug),Vancomycin (2ug), Amoxicillin (10ug), Erythromycin (15ug), Clindamycin (2ug), tetracyclin (30ug) and TMP (25ug). All according to the guidelines of Clinical and laboratory standard institute.

The *staph. aureus* ATCC25923 was prepared to test the quality of antibiotic susceptibility test as a positive control in the study.

Slide and tube coagulase test and DNAase test were done.

PCR For detection of mecA gene.

Detection of Biofilm Formation by MRSA: this was done by 2 methods:

a. **Culture of isolate on Congo red agar** for 24 hours at 37°C then the growth was classified into slim producer showing red or pink waxy colonies or non-slim producer showing smooth pink colonies with darkness at centre.
b. **By Microtiter tissue plate(MTP) Method**:20 ul of isolate were incubated in polystyrene plates with 96 wells at 37°C for 48 hours then washed using PBS then safranin and ethanol were added and read absorbance at 490nm wave length plate by ELISA reader. Negative control using trypticase soya broth media was used to determine background OD. The OD cut off value was separately calculated for each microtitre plate.

Molecular methods (genotypic): using Multiplex PCR:

Genomic DNA extraction: QiAamp DNA extraction mini kits were used for extraction of genomic DNA. Multiplex PCR for icaA ,icaD , eno ebps, and fib genes were performed in a final volume of 20ul by DFS master mix kits (Qiagene).Including Taq polymerase enzyme ,MgCl2 ,dNTP, (NH4)2SO4, Tris HCL and Tween -20. Reaction mixtures consisted of 12.5ul master mix, 1ul MgCl2, 25ul of each primer, 2ul distilled water and 1ul DNA template. Cycling conditions were primary denaturation at 94°C for 4min., denaturation at 94°C for 30s ,annealing at 52°C for 30s , extension at 72°C for 1min , then 30 cycle , followed by final extension at 72°C for 5 min. Detection of amplified product on agarose gel electrophoresis at 188, 192, 301, 180 ,and 405bp for IcaA, icaD,eno,ebps and fib genes respectively.

Table1: Forward and reverse primers designed for IcaA, icaD,eno,ebps and fib genes respectively.

Gene	Amplicon	Sequence
icaA	188	F:CAATACTATTTCGGGTGTCTTCACTCT R:CAAGAAACTGCAATATCTTCGGTAATCAT
icaD	192	F:TCAAGCCCAGACAGAGGGAATA R:ACACGATATAGCGATAAGTGCTGTTT
eno	301	F:AAACTGCCGTAGGTGACGAA R:TGTTTCAACAGCATCTTCAGTACCTT
ebps	180	ACATTCAAATGACGCTCAAAACAAAAGT CTTATCTTGACGCTTTATCCTCAGT
fib	405bp	GAATATGGTGCACGTCCACAATT AAGATTTTGACTTGAATCAATTTTTGTTCTTTT

Data analysis:

Pearson chi-square was used for statistical analysis. A P value less than 0.05 was considered significant.

RESULTS

Our study found a significant difference between the control and the patient groups as regard the prevalence of Staph. aureus. We found that 4/20 of control group had Staph. aureus (20%). And none of them were MRSA. As regard the patient group we found that 85% were S*taph aureus* while 15% were other bacteria and were excluded from our study. 33% (28) were MRSA proven by oxacillin disc and confirmed by presence of mec A gene while 57 /85(67%) were MSSA. MSSA were excluded from the study. All isolates of MRSA were sensitive to LZN & Targocid.27/28were sensitive to vancomycin & while sensitivity to amoxicillin, ciprofloxacin, tetracyclin, erythromycin& clindamycin and TMP were as follow: 90%,30%,20%,20%,14% and 10% respectively.

Phenotypic evaluation of biofilm formation **Table 2**:

Cut off value calculation	Ability to form biofilm	No	%
OD >4xODc	Strong	15	56
2xODc<OD<4xODc	Moderate	8	30
ODc<OD<2xODc	Weak	5	14
OD<ODc	Negative		

The MTP assay detected that among 28 MRSA isolates (n=15)56% was strong biofilm producers. While 30% (n=8) was moderate and 14% (n=5) was weak producers.(fig1)

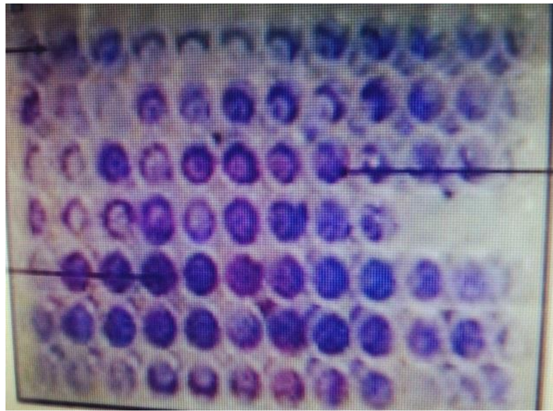

Fig. 1: phenotypic microliter plate assay

Table 3: Prevalence of MSCRAMMs encoding genes in relation to phenotypic expression of biofilm among MRSA isolates.

	Ica A	icaD	eno	ebps	fib
Strong	97%	100%	88%	22%	98%
Moderate	76%	88%	79%	18%	94%
Weak	71%	68%	78%	0%	92%

P value<0.05 significant

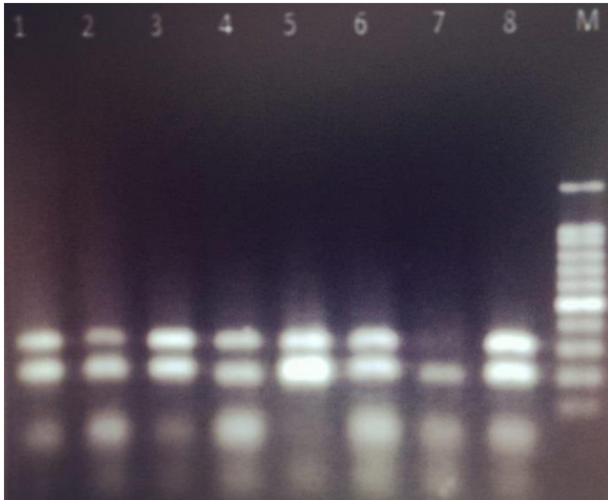

Fig. 2: Agarose gel electrophoresis of amplified PCR products

Stepladder marker from 100 to 1500bp of strong biofilm producers ica D at 192bp were found in 8lanes while eno at 301 bp were not found in lane 7

DISCUSSION

Our study found significant difference between both the patient and the control groups as regard the prevalence of *Staph. aureus* and MRSA and this was in accordance to Dean etal.This could be explained by that the anatomical deformity in soft palate facilitates the migration of *Staph.aureus* from the nose to the mouth cavity in children with cleft palates.

In our study we found 28/85 isolates were MRSA. All isolates were susceptible to LZN and targocid so they are proven to be effective in treatment of MRSA infections. However reduced susceptibility to LZN have been reported in other studies.9-10.In our study 27/28(96%) were sensitive to vancomycin and this disagreed with Mirazaee et al who found that sensitivity of MRSA to vancomycin was 100%.9.The present study found that fib gene was the most prevalent MSCRAMMs encoding genes among MRSA with expression 98%, 94 and 92% respectively among strong, moderate and weak Biofilm producer strain. Other studies found that ClfA and B genes were the most dominant in their expression among MRSA isolates11-13. This may be explained by the influence of epidemiological factors and the site of colonization meaning the type of sample. In these studies samples were nasal swab and skin swab from burnt skin.14-15. A strong correlation between the ability of biofilm production quantitatively and the gene expression exists in our work in accordance to other studies16-17.with respect to the difference in targeted gene.

CONCLUSION

MRSA harbor MSCRAMMs encoding gene family, There are several genes icaA&B&D & eno&ebps and fib are the targeted in our study. There is a strong correlation between ability of biofilm formation and its genotypic control.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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