Faculty of Veterinary Medicine
Department of Microbiology

"Molecular investigation of some virulence genes and antibiotic resistance of *Staphylococcus aureus* of animal origin"

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LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

VI. SUMMARY

A total number of 164 samples (mastitis milk, nasal swabs, wound swabs, abscesses′ contents) were collected from ruminant animals (cattle, sheep, and goat) either individually or from farms in Alexandria governorate and subjected to bacteriological examination.

Seventeen *Staphylococcus aureus* isolates were identified morphologically, biochemically (5 isolates from mastitis milk samples, 6 isolates from nasal swabs and 6 isolates from wound/pus samples).

Antibiogram profile of the isolates was carried out against 11 antimicrobials. 16/17 (94%) of isolates were resistant to at least one antimicrobial, 11/17(64.7) of them were resistant to three or more of antimicrobials (multi-drug resistance), highest sensitivity was observed for vancomycin and amoxicillin/clavulanic acid 17/17 (100%) while the highest resistance was observed for penicillin 14/17 (82.4%), and gentamicin 9/17(53%). Eight out of seventeen isolates (47%) showed resistance to oxacillin/cefoxitin (methicillin resistant *S. aureus*) all of them were multi drug resistant (MDR). i.e. MRSA strains are notorious for being multidrug resistant.

Among 17 clinical isolates of *S. aureus* isolated from collected samples, 1/17 (6%) was non biofilm producer and 16/17(94%) showed biofilm production, of them 7/17(40%) of isolates showed weak biofilm production, and 4/17(24%) showed moderate biofilm production, and 5\17 (30%) showed strong biofilm production.

The MDR *Staphylococcus aureus* isolates11/11(100%) were showing biofilm production, (3strong, 3 moderate and 5 weak) biofilm producers, while 5/6(83%) of the sensitive isolates were showing biofilm production (2 strong, 1 moderate and 2 weak) biofilm producers.

All the 17 isolates were examined for hemolysis by using three types of blood agar sheep blood agar, rabbit blood agar and horse blood agar for estimation of production of α, β, δ hemolysins. All the isolates were showing at least one type of hemolysin, 16/17(94%) of isolates were showing β hemolysin, 7/17(41%) were showing α hemolysin and 12/17(70%) of isolates were showing δ hemolysin.

Ten of MDR *S. aureus* isolates were molecularly screened for integron. Class 1 integron cassette were detected in 1/10 of tested isolates which exposed to further sequence

analysis reveals dihydrofolate reductase (*dfr*A15) gene cassette which encodes trimethoprim resistance, the GenBank accession number of the *dfr*A15gene sequences determined in this study is MW036489.

PCR was applied on 10 isolates of *S. aureus* representing all animal recovered isolates, and 2 isolates which were isolated from poultry (arthritis), all the isolates were MDR. The isolates confirmed as *S. aureus* by 23s rRNA PCR in all 12 isolates (100%).

The PCR assays for confirmation of the presence of 6 virulence genes (***agrI***, ***agrII***, ***agrIII***, ***agrIV***, ***tst***, ***lukED***).

agrI gene was detected in all the 12 isolates(100%), ***agrIII*** gene was detected in 10/12 (83%) of the isolates, ***agrII*** and ***agrIV*** genes were absent in all the isolates.

tst gene encoding(Toxin schock syndrome toxin) was detected in 7/12(58%) of isolates, ***lukED*** gene was detected in 11/12 (92%)of isolates.

The ERIC-PCR analysis showed that 12 *Staph aureus* isolates grouped into 6 Eric types (A1:A3, B1:B3) at 75% genetic similarity grouped into two main clusters (A &B).

The Eric type A1 contain one isolate(43SM) which were isolated from sheep mastitis, the Eric type A2 contain 2 isolates (71CW&90SN) which were isolated from cattle wound and sheep nasal swab respectively, the dissimilarity between the Eric type A1&A2 was 10%. The Eric type A3 contain isolates (26SW&52SW) which were isolated from sheep wound and cattle wound respectively.

The dissimilarity of the Eric types of cluster (A) was 14%.

The Eric type B1 contain two isolates (78CN&P11) which were isolated from cattle nasal swab and poultry arthritis respectively, the Eric type B2 contain two isolates, the Eric type B2 contain two isolates (02CM& P12) which were isolated from cattle mastitis and poultry arthritis respectively, the dissimilarity between the Eric type B1&B2 was 8%.

The Eric type B3contain 3 isolates (014CM, 21SW&8SP) which were isolated from cattle mastitis, sheep wound and sheep internal abscess respectively.

The dissimilarity of the Eric types of cluster (B) was 16%.

The dissimilarity between the Eric types of cluster A&B was 25%.

All the five MSSA isolates occur in the ERIC types of cluster (B)

All the isolates(5) in the Eric types of cluster (A) were MRSA, and only 2MRSA isolates occur in the ERIC types of cluster(B)

Various clusters generated in the ERIC-PCR dendrogram showed a 75% similarity of isolates from diverse origin. For example Eric type B2 contain two isolates (02CM& P11) which were isolated from cattle mastitis and poultry arthritis respectively, Thus, cross transmission of *S. aureus* isolates may occur in different animal species (cross-species transmission).