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Interspecific Diversity of Multidrug Resistant *Staphylococcus aureus* Isolates

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This study to estimate toxigenic diversity of three *S. aureus* strains (11SaUTI, 5SaRM and 21SaRW) isolated from clinical patient, food and water at Mesallata City, Libya. As well as to know antibiotic resistance, phage typing, biofilm formation and genoypes (*icaA* gene and 16s rDNA gene). Three *S. aureus* isolates were selected from isolates obtained from the different sources and identified by conventional biochemical tests and confirmed by VETIk kit. These isolates exhibited variation of toxigenic, antibiotics resistance, biofilm formation and phage plaque forming (phage typing). The amplified 16s rDNA gene to confirm the diversity of *S. aureus* isolates . In addition to molecular diagnosis to have a complete and clear image for bacterial etiology based on genetic variability. DNA genome of *S. aureus* isolates was used as template for PCR to amplify 16s rDNA gene. Partially, length of 16s rDNA gene could be synthesized using the internal primer sets combination in PCR to confirm its interspecific to *S. aureus* with an expected size 1219 bp. The replacement situation of nucleotide sequences were with percent of camera 1.067, 2.217 and 1.313 respectively. Three *S. aureus* isolates were recorded in gene bank under accession no. LC121093.1 (11SaUTI); LC121094.1 (5SaRM) and LC121095.1 (21SaRW).

Keywords: *S.aureus*; Antibiotic resistance, biofilm, phage typing, *ica* A gene and 16s rDNA genes, PCR.

The pathogenicity of *S.aureus* defined many years back[28]. It causes abscesses, boils, conjunctivitis especially in newborn, cross-infections in hospitals septicemia, and mastitis[26]. *S.aureus* is described as a variable bacterium with many morphological variants[20]. Development of antibiotic resistance *S. aureus* strains which is a serious setback in many hospitals causing various hospital outbreaks has been reported in many studies[2]. It is essential to characterize accurately the extent of genotyptic and phenotypic variation present in the pathogen population. There are various conventional methods (biotyping, antibiotyping and phage typing) which can be used to identify and characterize these organisms[3]. But application of PCR based 16sr DNA, biofilm primers has been played a major tool in finding out the relationship between the various species of microorganisms[11].

Construction of dendrogram using similarity matrix gives the relationship between the isolates. Earlier report on similarity matrix of *S. aureus* by suing 16s DNA primers has shown dissimilarity between the organisms present in the sputum of various infected persons[29]. The present study was to isolate, and analyze the genotypic

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diversity among three *S.aureus* collected from different sources at Mesallata, Libya. As well as to know isolates pathogenesis; prevalence; antibiotic resistant; phage typing; phenotypic of biofilm formation, *icaA* and 16s rDNA genes detection.

MATERIALS AND METHODS

Bacterial isolation

S. aureus strains were isolated on Baird Parker agar from clinical patient (urinary tract infection), food (raw milk) and water (raw water) at Mesallata City, Libya. Black, shiny and convex colonies surrounded by clear zone were isolated and subjected to methicillin sensitivity test. Two methicillin resistant *S aureus* (5SaRM and 11SaUTI) and one methicillin sensitive (21SaRW) were selected from bacterial isolates obtained. These three bacterial isolates were subjected to gram stain and conventional biochemical tests[19] for identification and confirmed by using VETIC kit (Bio Mèrieux, France)[9].

Coagulase and DNase test

Coagulase production was determined in 10^{-1} dilution of both human and sheep plasmas in test tubes. Result interpretation was described by Collins and Lyne[10] and Umoh[30]. DNase production was done on DNase agar (Difco) reconstituted according to the manufacturer's instruction and following the procedures as described by Collins and Lyne[10].

Antibiotics resistant test

S. aureus isolates were tested against antibiotics using the disc diffusion method and interpreted according to Clinical Laboratory Standards Institution[7]. and confirmed by the broth dilution method, which determined the minimum inhibitory concentration (MIC)[7].

Detection of Biofilm Formation

Three *S. aureus* isolates (10^9CFU/ml^{-1}) were cultured in 96-well micro titer plates (BHI) and incubated at 37°C for 48h. The inoculated plates were aspirated and wells were washed with phosphate buffer saline (PBS). The plates were stained with 0.5% crystal violet for 5 min. then washed with tap water and 200µL of 95% ethanol was added. The biofilm formation was considered positive when an optical density at 570nm was equal or more than 0.2 OD[31].

Isolation of *S. aureus* phages

The lysate phages for purified *S. aureus* cultures were isolated from hospital waste water. The lysate phages were enriched on double strength nutrient broth using purified *S. aureus* as host phages. Filtrated phages were determined by seeding agar overlay method according to Adams[1]. Three isolated phages were produced by inoculating log phase bacterial culture (approximately 1×10^8 CFU ml^{-1}) in nutrient broth with multiplicity of infection varying between 0.01 and 1.0. Bacterial debris and survivors were removed by centrifugation at 6000 rpm for 10 min. each suspension was diluted to make at least 10^9 PFU ml^{-1}.

Phages morphology

Transmission electron microscope (TEM) was used to detected phages using negative staining method with 1% aqueous uranyl acetate. The grids were air dried and were examined by TEM (JEOL – JEM – 1010 Electron microscope) according to Heringa[18]. In the Regional Center for Mycology Al-Azhar Univ. Each suspension phage was tested against three *S. aureus* isolates by plaque assay according to Adams[1].

Detection of *icaA* regulator gene using PCR

The genomic DNA of *S. aureus* isolates was extracted as follow. Approximately 2×10^9 CFU/ml of each isolate was washed in 1mL of sterile distilled water and then pelleted by centrifugation at 6.000 rpm for 5 min. Each pellet was suspended in 100 µl of sterile distilled water and then boiled in a water bath for 30 min to release the DNA in solution[4].

PCR amplification of the *icaA* gene

Responsible for biofilm production by investigated *S. aureus* isolates gene was performed using primer sets (forward *icaA* F 5-TCTCTTGCAGGAGCAATCAA-3 and reserve, R: *icaA* R5- TCAGGCACTAACATCCAGCA-3) with expected 188 bp according to Arciola[4]. The PCR reaction mixture consisted of 250ng of DNA; 20 pmol concentration of each PCR primer; 0.2 µ1 of concentration of dNTPs, 2.5m mol/1MgCL2 and 2.5 U of Ampli Taq DNA polymerase in 1× reaction buffer. The samples were subjected to initial denaturation at 95°C for 2min, A 30 cycles of amplification consists of denaturation (94°C for 45 s) Annealing (63°C for 45 s) and Extension (72°C for 1min)[16].

PCR amplification of the 16srDNA gene

The alignment of the rpo B sequences of *S. aureus, S. lugdunensis, S. intermedius, S. saccharolyticus*, and *S. caprae* enabled us to design consensus PCR primers 2491F (5-AACCAATTCCGTATIGGTTT-3; base positions 2491 to 2511) and 3554R (5-CCGTCCAAGTCATGAAAC-3; base positions 3554 to 3573) according to Drancourt and Raoult[13]. To amplify a 1.081-bp variable fragment in 27 additional Staphylococcus species under investigation. All PCR mixtures contained 2.5× 10-2U of Taq polymerase per µ1 × Taq buffer, 1.8 mM MgCl2 (Gibco BRL, Life Tecnologies, CergyPotoise, France), 200 µM concentrations of dNTPs, and dCTP (Boehringer Manheim GmbH, Hilden, Germany), and 0.2 µM concentrations of each primer (Eurogentec, Seraing, Belgium). The samples were subjected to 35 cycles of denaturation at 94°C for 30 s, primer annealing at 52°C for 30s and de novo DNA extension at 72°C for 60s. The PCR product was electrophoresed on 1% agarose gel to determine the size of the product fragments and visualized by ethidium bromide staining.

Purification of PCR product

The PCR fragment of 16s rDNA gene was excised from the gel and purified using a QIA quick gel extraction kit **(Qiagenic; Germany)**.

Sequence analysis

Assembly and analysis of sequences and generation of the nucleotide sequence alignment with representative reference FAdVs strains' hexon sequences from each genotype previously published[6]**.** retrieved online from public database (http:// www. ncbi. nlm. nih. gov/) were performed suing Bio Edit (tom Hall, Ibis biosciences, Carlsbad, CA) and Clustal W multiple alignment method.

Fig. 1. Baird-Parker Egg Yolk Tellurite agar cultured with three *S. aureus* which appeared as black shiny convex colonies with clear zone surrounding colonies

Phylogenetic tree analysis

The subsequent phylogenetic analysis was implemented by using MEGA version 6.0 constructed by Maximum Likelihood method based on the 590 bp region corresponding to the 16s rDNA gene was used to confirm the clustering of the different species according to their 16sr DNA loop sequence.

RESULTS

Bacteria isolates

Three *S. aureus* isolates (11SaUTI, 5SaRM and 21SaRW) showed variation of growth on Baird Parker agar medium. Isolate No 11SaUTI and isolate No 5SaRM gave strong growth while isolate No 21SaRW exhibited low growth (Fig 1). Qualitative analyses were used to specific media to isolate the colonies of potentially default pathogens Namely (11SaUTI), (5SaRM) and (21SaRW) and confirmed according to Bergey's Manual of Determinative Bacteriology and by VETIC kit.

Three *S. aureus* isolates (11SaUTI, 5SaRM and 21SaRW) showed variation of toxigenic potential based on coagulase test (HP, SP and SP+HP) and DNase test. The isolates classified into three categories; whereas the isolates (11SaUTI and 5SaRM) showed Strong toxigenic on HP and HP+SP; while moderate toxigenic on SP. The isolate (21SaRW) gave moderate toxigenic on HP and HP + SP; while low toxigenic on SP. On the other hand the three isolates showed strong toxigenic potential DNase test. The obtained results proved that three isolates could be involved of potential pathogenic.

Antibiotic susceptibility

Methicillin resistant *S. aureus* isolates (5SaRM and SaUTI) and methicillin sensitive *S. aureus* isolate (21SaRW) showed variation to other antibiotic sensitivity as shown in table (2). Both isolates (MRSA and MSSA) are sensitive to vancomycin and resistant to ampicillin, tobramycin and erythromycin.

Biofilm formation of *S. aureus* isolates

A biofilm positive phenotype was defined as OD ≤ 0.17 at 570nm. Interpretation of biofilm production, strong biofilm formation was classified as 0.7 to 1.0 (OD 570nm), moderate biofilm formation was classified as 0.3 to 0.4(OD 570nm) and weak

biofilm ≤ 0.3(OD 570nm).

The results presented in Table (3): Showed that (5SaRM) and (21SaRW) isolates exhibit strong biofilm formation with optical density 1.5 (OD) and 1.1 (OD) respectively. While, the clinical isolates showed moderate biofilm formation with 0.47 (OD).

Regarding the distribution of biofilm production among antibiotics sensitivity of *S.aureus* isolates found that no relationship whereas (21SaRW) isolate was MSSA and strong biofilm formation, (11SaUTI) was MRSA and moderate biofilm formation and (5SaRM) isolate was MRSA and strong biofilm formation.

S. aureus phages

The phages against three *S.aureus* isolates were formed plaques lysate on double layer culture plate which showed variation of plaque morphology. (Diameter, clear circular plaques) (Table 4).

Phage morphology

Transmission electron microscopy (TEM) was observed morphology phages lysate against *S.aureus* isolates fig. (2). The shape and dimensions of phage was possessed isometric hexagonal head and contractile tail. The. The dimension of the head was ranged 65×82 and dimensions of the tail type were 75.5×121 nm. Consequently. The phages were classified as belonging to family *Myoviridage*.

Phage typing

The table (4) illustrated the phage typing of three *S. aureus* isolates. It was found that phage specific (11SaUTI) isolates reacted high lysed with (11SaUTI) isolate and moderate lysed with (5SaRM) isolate and nil lysed with (21SaRW) isolate.

Molecular detection of biofilm gene (*icaA*)

The purified total DNA of *S. aureus* isolates (11SaUTI), (5SaRM) and (21SaRW) were

Table 1. Variation of toxigenic three isolates of three *S .aureus* isolates

Source of isolates	Code	Coagulase			DNase
		Hp	Sp	Hp+Sp	
Food (raw milk)	5SaRM	++++	++	++++	++++
Human (urinary tract infection)	11SaUTI	++++	++	++++	++++
Water (raw water)	21SaRW	++	++	++	++++

Hp = Human plasma, Sp = Sheep plasma, Strong (++++), Moderate (++), Low (+)

Table 2. Multidrug resistance profile of pathogenic bacteria against individual antibiotics

Test group	Antimicrobial agents	Dose	*S .aureus*		
			21SaRW	5SaRM	11SaUTI
Beta- lactams	Methicillin- ME	5µg	SR	RR	RR
	Ampicillin- AM	10µg			
Cephalosporins	Cefotaxime-CTX	30µg	RSI	RSS	SSR
	Cefodizime-CDZ	30µg			
	Cefazolin-CZ	30µg			
Aminoglycosides	Gentamycin- CN	10µg	RRR	IRR	RRS
	Tobramycin-TOB	10µg			
	Kanamycin-K	30µg			
Rifamycins	Rifamycin-RF	30µg	S	S	R
Glycopeptides	Vancomycin- VA	30µg	S	S	S
Macrolides	Erythromycin-ERT	15µg	R	R	R

R = resistant, I = intermediate sensitive, S = sensitive

confirmed by agarose 1.5% (fig.6-A), UV spectrophotometer where 1.8, 1.5 and 1.7 260/280 ratio OD and the DNA concentration were 80,95 and 75µg/5gm cell tissues respectively.

The PCR technique was applied to the detection of *icaA* gene biofilm in three *S. aureus* (11SaUTI), (5SaRM) and (21SaRW) isolates. It was noticed the presence of *icaA* gene in biofilm producing in *S.aureus* isolates with fragment at 250 bp in (11SaUTI) and (5SaRM) as well as (21SaRW), *icaA* gene was found with a different molecular size band at 230-bp as show in fig. (3).

Table 3. Biofilm formation assay of *S. aureus* from different sources using (Tissue culture plate method by ELISA reader)

Optical density (O.D.) measurements	*S. aureus* isolates		
	11 SaUTI	5SaRM	21SaRW
Growth (O.D. 620 nm)	0.9	1.2	1.4
* Negative Control (O.D. 620 nm)	0.11		
Biofilm (O.D. 570 nm)	0.47	1.5	1.1
Biofilm production category	Moderate	Strong	Strong

* Negative Control = non inoculated medium

Table 4. Plaque characteristics of the isolated *S. aureus* phages

Phages specific infection *S .aureus* isolates						
Phage isolates	Phage diameter (mm)	Plaque Type	Plaque edge shape	Lysate phages 11SaUTI	5SaRM	21SaRW
11SaUTI	2	Clear	Regular	++++	++	—
5SaRM	1	Turbid	Irregular	++	++++	+
21SaRW	4	Clear	Regular	—	++	++++

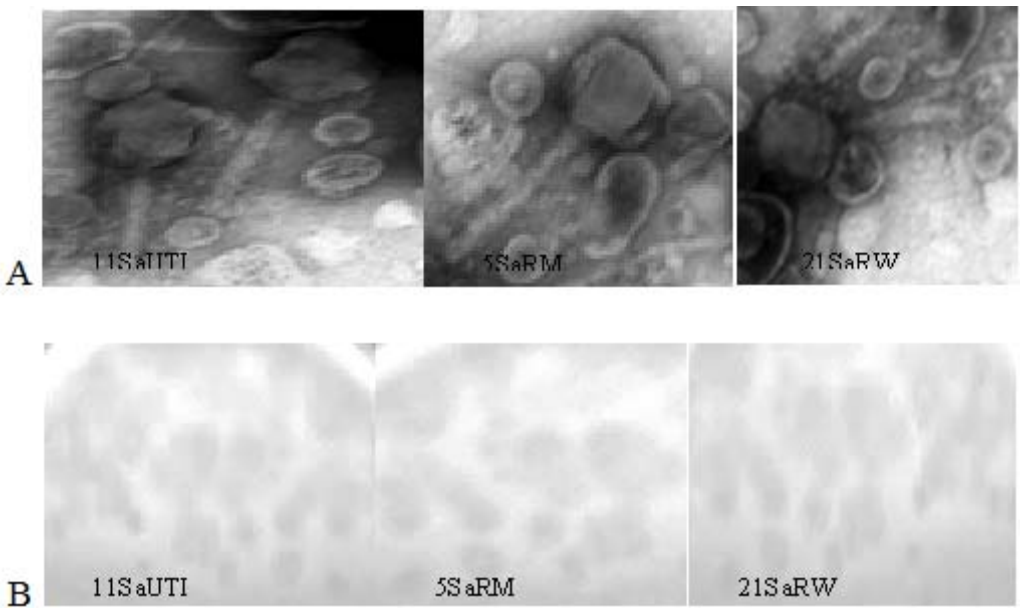

Fig. 2. Electrophotogram of phages for three *S. aureus* isolates, (A) TEM shown different phage typing for *S. aureus* and (B) different type plaque assay showing different type of plaque morphology

Molecular characters of 16s rDNA gene

16s rDNA gene of three *S.aureus* isolates was formed Contag. (one of each isolate) (fig.4) with diversity of the partial nucleotide sequence. The DNA amplicons for three isolates showed 1218 bp but then varied in density fragment and sequences. The resulted sequences of three isolates were aligmened as well as with *S. aureus* recorded in the GenBank using DNA MAN program which identified as *S. aureus*. On the level of DNA sequencing of 16s rDNA gone, the similarity between three present sequences belonging to two groups could be summarized as follows.

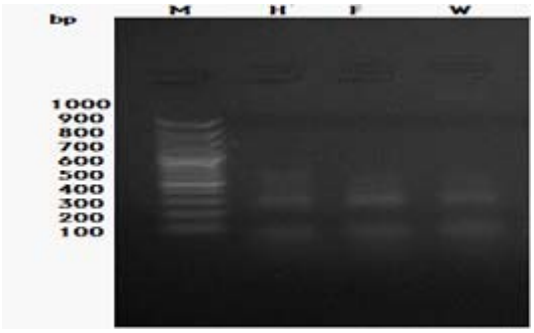

Fig. 3. Electrophotogram of agarose gel (1.5%) showing PCR products amplified fragment of *icaA* gene. Lane M, Trackit™ 100bp DNA leader; lane (11SaUTI); (5SaRM) and (21SaRW) PCR of *S. aureus* isolates

Table 5. Morphological characters of *S. aureus* phages particle size

Phage	Head diameter (nm)	Tail length (nm)	Family
11SaUTI	75	105	*Myoviridage*
5SaRM	65	121	*Myoviridage*
21SaRW	82	75.6	*Myoviridage*

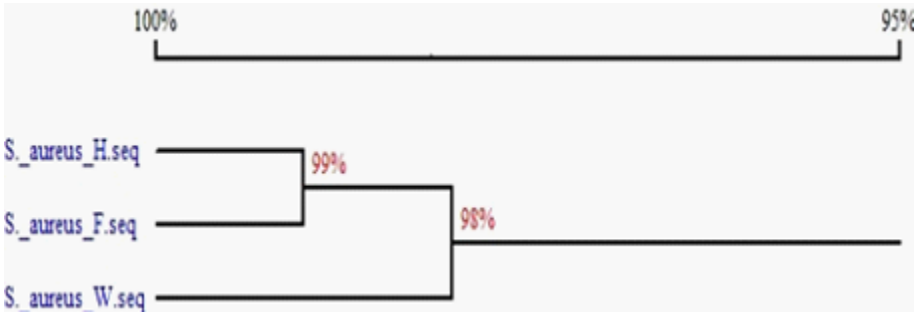

Fig. 4. Phylogenetic tree of 16s rDNA gene for three *S. aureus* isolates (11SaUTI, 5SaRM and 21SaRW) based on nucleotide sequences. The dendrogram displaying the percentage similarity of sequence homology among isolates

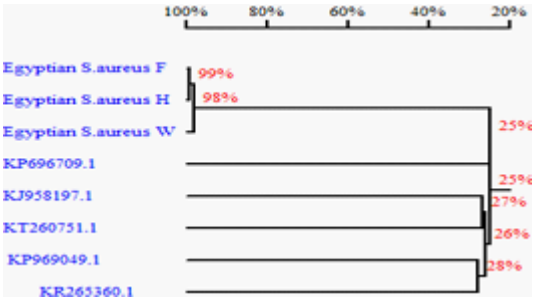

Fig. 5. Phylogenetic tree representing the relationship between the three isolates and Egyptian isolates 2 based on DNA sequence homology

In the first group include of *S. aureus* isolates (11SaUTI-$_{H\text{-}Seq}$) and (5SaRM-$_{F\text{-}seq}$) with similarity 99%. Regarding the second group, that contains the fist group and *S. aureus* (21SaRW-$_{W\text{-}Seq}$) with similarity 98% of 16s rDNA gene fig. (4).

One the level of 16srDNA gene sequences the similarity between three present isolates compared with *S.aureus* stains recorded on Genbank. Sequences belonging to two major group. The first major group in could three present isolates and the sacand major group (KP696709.1 and KR 265360.1) with 25% similarity fig. (5).

Nucleotide diversity

The three *S.aureus* isolates showed great similarities in the nucleotide sequences with same differences in some nitrogen bases as shown in Table (6). The nucleotide sequences of 16s rDNA gene were revealed situation replacements among three *S. aureus* isolates as shown in Table (6). The replacement percentage of nucleotide sequences cumera among three *S. aureus* isolates (11SaUTI, 5SaRM and 21SaRW) was with percent of cumera 2.29%.

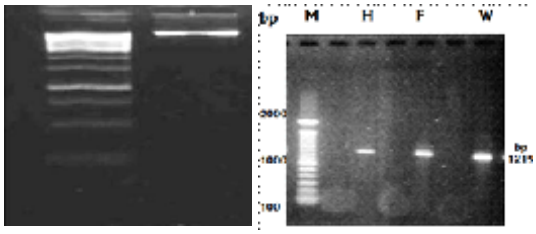

Fig. 6. Electrophotogram of agarose gel (1.5%) showing**: (A)** total genomic DNA isolated from three *S. aureus* isolates (11SaUTI, 5SaRM and 21SaRW and **(B)** PCR products amplified fragment of 16s rDNA gene. Lane M, Trackit™ 100 bp DNA leader; lane H; F and W PCR of *S. aureus* isolates

Data in table (7) showing the cumera percentage between three *S.aureus* isolates. The cumera percentage between (11SaUTI) and (5SaRM) was 1.067, (11SaUTI) and (21SaRW) was 2.217 and (5SaRM) and (21SaRW) was 1.313.

16s DNA secondary structure production

The 16srDNA partial gene primary sequences of the three *S.aureus* isolates were nearly identical, but secondary structure of three isolates was exhibited significant variations. The Secondary structures of 16s rDNA gene were inferred on the basis of the base pairing models conducted by DNAMAN software. Variations in inferred secondary structure depend on number of loops such as hairpin loops, bulge loops, internal loops, bifurcations and sticks (nucleotide pairs, A-U or G-C) differentiated between the three isolates fig. (7). The dominating secondary structure required the free energy (DG) for folding of primary structures into secondary structures were calculated as follow: -146.89 kcal/mol for (11SaUTI), -142.3kcal/mol for (21SaRW) and -147.36 kcal/mol for (5SaRM) isolate .

Table 6. Replacement situation of nucleotides in 16s rDNA gene sequence for three *S. aureus* isolates

S .aureus Isolates	Situation of nucleotides											
	10	20	36	51	56	65	71	72	77	83	116	118
11SaUTI	G	A	G	T	T	T	A	A	G	C	T	C
5SaRM	C	C	C	C	A	T	A	A	G	T	T	C
21SaRW	C	C	C	C	A	G	T	T	A	C	C	T
	160	162	502	542	593	634	683	712	737	826	912	998
11SaUTI	G	A	T	G	A	G	A	G	C	A	G	G
5SaRM	A	G	T	G	A	G	A	G	C	A	G	A
21SaRW	A	G	G	A	C	T	C	A	G	C	C	A
	1010	1015	1030	1101								
11SaUTI	T	A	A	C								
5SaRM	G	T	G	T								
21SaRW	G	T	G	T								

Table 7. Matrix showing the cumera percentage between *S. aureus* isolates

S. aureus isolates	11SaUTI	5SaRM	21SaRW
11SaUTI	___	1.067	2.217
5SaRM	1.067	___	1.313
21SaRW	2.217	1.313	___

DISSCUSSION

S. aureus globally has become a major clinical problem. In an effort to develop effective control strategies against the genotypically variated organisms, it is essential to characterize accurately the extent of genetic and phenotypic variation present in the pathogen population. There

Fig. 8. Secondary structure for *S. aureus* present isolates 16s rDNA partial sequence

are various conventional methods (biotyping, antibiotyping and phagetyping) which can be used to identify and characterize these organisms.

Three *S. aureus* isolates obtained from clinical patient (11SaUTI), raw milk (5SaRM) and raw water (21SaRW). These isolated were cultured on specific medium Baired Parker agar medium. Three isolates were adapted in their habitats. *S.aureus* isolated from milk and cheese[24], *S.aureus* isolated from UTI in patent[17] and *S.aureus* isolated from water[27].

The three *S.aureus* isolates differed in toxigenic potential based on coagulase test (HP, SP and SP+HP) and DNase test. The three isolates where classified into three categories; whereas (5SaRM) isolate showed height toxigenic on HP, SP and HP+SP while moderate toxigenic on SP. (11SaUTI) isolate showed Strong toxigenic on HP and HP+SP; while moderate on SP and (21SaRW) isolate showed moderate toxigenic on HP and HP+SP; while low toxigenic on HP. On the other hand the three isolates showed Strong toxigenic potential on DNase test. The obtained results proved that three isolates could be involved of potential pathogenic. The present work evaluates tube coagulase test, slide coagulase test, and Slidex Staph plus test for *S. aureus* detection considering coagulase gene PCR as the reference method.

A variety of three isolates are considered major causes of milk borne illnesses worldwide. Milk understandably an important constituent of human diet and raw milk is an ideal growth medium for several microorganisms. Milk and its derivate are considered vehicles for *S .aureus* infection in human[33]. In dairy cattle, *S. aureus* is frequently associated with subclinical mastitis and may contaminate milk and other dairy products[25].

Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food[23]. *S. aureus* has been reported to frequently show multiple antimicrobial resistance patterns[14].

The obtained results showed that human (11SaUTI) and food (5SaRM) isolates indicated the methicillin resistance (MRAS) due to multi exposure to methicillin drug through therapy of patient, while water (21SaRW) isolate was sensitive (MSSA) due to no exposure methicillin drug. On other hand the three isolates showed variation in other antibiotic sensitivity. Diversity in the antibiotic susceptibility patterns of *S. aureus* isolates was reported[12,14]. Furthermore, impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

The DNA extracted from all confirmed *S. aureus* did not yield any PCR products with all the primers used for detecting methicillin-resistance genes. Similar results reported[21]. From the previous result no relation between biofilm formation and resistance to methicillin antibiotics

The tested *S. aureus* (11SaUTI), (5SaRM) and (21SaRW) isolates exhibit strong biofilm formation with optical density 1.5 and 1.1(OD) respectively. While, the (11SaUTI) isolate showed moderate biofilm formation with 0.47 (OD). Regarding the distribution among antibiotics resistant of *S. aureus* isolates, *S. aureus* (11SaUTI) that showed moderate biofilm production was MRSA and (21SaRW) that was strong biofilm producing isolate was antibiotics sensitive. All *S. aurues* were tested for the presence of *icaA* gene biofilm. It was found that three isolates human, food and water were found to be positive for gene, giving a 200- to 250 bp band for *icaA* gene.

Microorganisms were observed attach to surfaces and develop biofilms[5,22]. Biofilm-associated cells can be differentiated from their suspended counterparts by generation of an

Extracellular Polymeric Substance (EPS) matrix, reduced growth rates, and the up- and down-regulation of specific genes. Attachment is a complex process regulated by diverse characteristics of the growth medium, substratum, and cell surface. An established biofilm structure comprises microbial cells and EPS, has a defined architecture, and provides an optimal environment or the exchange of genetic material between cells. Cells may also communicate via quorum sensing, which may in turn affect biofilm processes such as detachment.

The phages lysate against three *S. aureus* isolates formed different plaques morphology (diameter, clear circular plaques) shown on double layer culture plate. Morphology of phages lysate against *S. aureus* isolates were observed by TEM. The shape and dimensions of phage possessed isometric hexagonal head and contractile tail. Consequently, the phages were classified as belonging to family Myoviridae (group A). It was found that phage specific to (11SaUTI) isolate reacted high lysed with (11SaUTI) and moderate lysed with (5SaCHR) isolate and nil lysed with (21SaRW) isolate.

Temperate bacteriophages play an important role in the pathogenicity of *S. aureus*, for instance, by mediating the horizontal gene transfer of virulence factors[8]. Here we established a classification scheme for staphylococcal prophages of the major Siphoviridae family based on integrase gene polymorphism. Bacteriophages have a tremendous impact on the biology of their bacterial hosts, because they play an important role in bacterial ecology, evolution, and adaptation. For instance, in the human pathogen *S.aureus*, prophages are responsible for the emergence and evolution of new threatening strains such as the community-acquired MRSA strains which carry PVL encoding prophages. Despite their importance, comprehensive picture of the distribution of prophages in the *S. aureus* strain populations was lacking.

16s rDNA gene of three *S. aureus* isolates was formed Contag (one of each isolate) and showed the partial nucleotide sequences (1219 pb). The resulted sequence were compared between three *S. aureus* isolates as well as with *S. aureus* recorded in the Gen Bank using DNA MAN program and identified as *S. aureus*. On the level of DNA sequencing of 16s rDNA gone and the similarity between three present sequences belonging to two groups. The 16s rDNA partial gene primary sequences of the three *S. aureus* isolates were nearly identical, but secondary structure of three isolates were exhibited significant variations in their secondary structure by DNAMAN software. Variations in inferred secondary structure depend on number of loops such as hairpin loops, bulge loops, internal loops, bifurcations and sticks (made up of a sequence of nucleotide pairs, A-U or G-C) differentiated between the three isolates as well as the free energy (dG) required for folding of primary structures into secondary structures were calculated as follow: -146.89 kcal/mol for *S.aureus* (11SaUTI), this was followed by *S.aureus* (21SaRW) (- 142.03Kcal/mol) and *S. aureus* (5SaRM) (-147.36 Kcal/mol) both of *S.aureus* (11SaUTI) and *S. aureus* (5SaRM) were summarized in free energy, while *S. aureus* (21SaRW) differ than previous isolates.

To develop a rapid and accurate method of typing large numbers of clinical isolates of *S.aureus*, the spacer region C of the rRNA operon (1391-507) (16S23S)1 was enzymically amplified from 322 strains[32]. When the products were separated by denaturing PAGE, 15 variable – length m, alleles were demonstrated, ranging in size from 906 to 1223 bp. The variable length Hpall-digested region C (region E; 1446-196(16S, 23S)1 amplification products were cloned into M13mp18RF to sequence separate variable- length alleles. A total of 17 region E inserts were sequenced, aligned and divided into nine alleles by length (938-1174) and sequence properties. The 165-23s spacer rDNA varied in length (303-551bp) and in properties; three alleles contained at RNA Ik gene alone, two alleles contained a tRNA". And a tRNA Ak gene, and four alleles lacked tRNA genes. The sequences of two alleles showed less than 10/0 variation when isolated from two or three *S. aureus* strains. The 48 penicillin and methicillin-sensitive strains were divided into 26 riboytpes; in contrast, the 274 methicillin-resistant *S. aureus* (MRSA) strains were divided into nine riboytpes (A-I) with 97% typing as either ribotype A or B (mnl was missing in B). The sequence conservation of the mn operons argues for the use of the 165-23s spacer region as a stable and direct indicator of the evolutionary divergence of *S.aurues* strains.

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