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MOLECULAR CHARACTERIZATION OF *STAPHYLOCOCCUS* SP. ISOLATED FROM DIFFERENT TYPES OF CHEESE

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Abstract – Cheese production is one of the great Eastmain industries. Among all types of cheese, kariesh, industrial white, old and roomy cheese were investigated as a source of isolating *Staphylococcus* sp. Antibiotic susceptibility test of 7 antibiotics was tested for the *Staphylococcus* as evaluated. The selected bacterial isolates were proved to be resistant to Penicilin(P), Chloramphenicol(C), Ofloxacin (OFX), Cefepime (CPM), Meropeneme (MEM), Metronidazole (MTZ), Doxycycline (DO), Ceftazidime (CAZ), Clindamycin (DA), Rifampine (RA), Azetreonum (ATM), Tetracycline (TE) and Trimethoprim-sulfamethoxaz (STX). Molecular identification were approved to confirm the suspected *Staphylococcus* sp. Molecular studies of total DNA showed that all the selected bacterial isolates have plasmids with size less than 5000bp. Molecular characterization were evaluated using specific PCR (12 pair of primer). Detection of multidrug resistance gene among the selected *Staphylococcus* sp. isolates were evaluated. Data revealed that TEM gene present in all *Staphylococcus* isolates, on the other hand (CIT and VIM genes) present in bacteria isolated from white cheese only. *Staphylococci* isolated from white cheese have KPC, SHV and OXA55 genes. Mececylline gene (Mec) and IPM gene were absent in all tested *Staphylococcus* isolate, although *Staphylococcus* isolated from (industrial white cheese, old cheese and roomy cheese) have vancomycin gene (VAN).

INTRODUCTION

Cheese is a product derived from milk that is manufactured in a varied of tastes, qualities and forms (Fankhauser, 2007). Types of cheese are collected or categorized according to conditions of process such as fermentation interval, quality, approaches of production, fat %, animal milk, nation or state of beginning, etc (Fox *et al.,* 2000). Cheese is an important nutritional category of human food. However, fresh white cheese is often prepared with unpasteurized milk and put in public markets. This can raise the possibility of contamination with pathogenic bacteria. The occurrence of multidrug-resistant pathogenic bacteria in nutrition is a vital public health alarm (De la Rosa-Hernández *et al*., 2018).

Food poisoning by *Staphylococcus* is one of common global food-borne diseases. Toxin secreted by *Staphylococcus* reason of disease in about 241,000 personnel in the U.S. annually (Yilma *et al*., 2007). *Staphylococci* correlated disease is amongst severe public-health problems and the second record affluent diseases to necessity in developed country. Conferring to WHO staphylococcal associated food borne disease are the second most public nutrition allowed disease subsequent to *Salmonella* (Shimelis *et al.,* 2018).

S.aureusis commonly food born pathogenesis of great importance for animal and human concern. The infection is mainly transmitted through food chain pathway starting from milking till manufacturing of dairy products. *S.aureus* is an important food pathogen that is liable to contaminate dairy products kariesh cheese and ice cream from different sources during their production, processing and handling that make them unfit for human consumption or even a dangerous source of infection among consumers constituting a potential health hazard. This can be occurred under certain conditions during production as well as when they are cut and

packaged for consumption (Elmaghraby *et al*., 2018). Utmost pathogenic bacteria that are generally convoluted in causing disease to human presences and animals presented extensive amount of resistance to generally used antibiotics (DACA, 2009). *Staphylococcus aureus* is capable of producing an expansive repertoire of cell surface-associated and extracellular virulence factors in human where it is one of the focal human pathogen (John *et al.,* 2012). *Staphylococcus* is one of family Staphylococcaceae. They look Gram-positive cocci and arrangement in groups below microscope. They are facultative anaerobic organisms, Originate worldwide (Madigan *et al*., 2005).

The presence of *S. aureus* in milk was variable in different regions, and these variations may be due to season, number of animals on the farm, farm size, hygiene status, variation in sampling, farm management practices, geographical location, and differences in detection methods and variation in types of samples evaluated (El-Sayed *et al.,* 2011).

Staphylococcus includes at least 40 species. Most are inoffensive for human and other organisms. *Staphylococcus* microbe has been created influid living (Harris *et al.,* 2002). *S. aureus* (strain LAC, SSR42) is required for wild-type levels of erythrocyte lysis, resistance to human and pathogenesis in skin and soft tissue (John *et al.,* 2012). The pathogenicity of *Staphylococcus aureus* is shaped by a variation of extracellular and proteins of cell wall-associated (Bronner *et al*., 2004 and Cheung *et al*., 2004). The expression of these virulence factors is controlled by a complex network of global regulatory elements (Kim et *al.,* 2014).

MATERIALS AND METHODS

Study period and area

The present study was carried out in 2018 at different Alexandria localities. Type and source of samples differ where different local cheese sample were used. White cheese, industrial white cheese, old cheese and roomy were investigated. Sampling was carried out in sterile containers.

Isolation of staphylococcus isolates

All pure culture bacterial isolates were tested for growth in mannitol salt agar and incubated at 37 °C for 24-48 hours. Staphylococci appeared as yellow colonies and regarded as confirmative identification of Staphylococci (Shimelis *et al.,* 2018). All cultures of *Staphylococcus* species were examined using Gram's stain.

Molecular identification of bacterial isolates

Pellets of bacterial cells were used for DNA extraction using Gene JET Genomic DNA Purification kit. The region of 16S rRNA was amplified using the universal primers (F: AGAGTTTGATCMTGGCTCAG and R: TACGGYACCTTGTTACGACTT) (14). The reaction was implemented using DNA template. Sequences of the 16S rRNA genes were obtained from the NCBI database. Multiple alignments based on the most closely related sequences similarity by using the BLAST program1. A phylogenetic tree was reconstructed using the Bioedit software.

Determination of the resistance using molecular techniques

Pellets of bacterial cells were used for plasmid extraction using GEBRI kit. Plasmid purity and concentration were measured using a Nano Drop™ spectrophotometer.

Detection of virulence genes

In the present work the tested genes (8 pairs) were used and listed in Tables 1 for different virulence gene.

RESULTS

All pure culture isolates were tested for growth in mannitol salt agar. All the colonies with yellow halo were selected as confirmative identification of staphylococci. The examination of four *Staphylocoous* strain represent to each type of cheese. All selected *staphylococcus* isolates were confirmed by different biochemical reaction (urease, catalase, MR, gelatinase and citrate). *Staphylococcus* ASZ12 was isolated from white cheese, *Staphylococcus* ASZ21 was isolated from industrial white cheese, *Staphylococcus* ASZ31 was isolated from old cheese and *Staphylococcus* ASZ37 was isolated from roomy cheese.

Antimicrobial resistance of the selected *staphylococcus* isolates

All *staphylococcus* selected isolates were resistant to Penicilin(P), Chloramphenicol(C), Ofloxacin (OFX), Cefepime (CPM), Meropeneme (MEM), Metronidazole (MTZ), Doxycycline (DO), Ceftazidime (CAZ), Clindamycin (DA),

Table 1. Primer sequence used for screening of virulence genes coding KPC, TEM, VIM, MEC, CIT, IMP, SHV and VAN

Gene	Sequence	Reference
KPC	F: CGTCTAGTTCTGCTGTCTTG, R: CTTGTCATCCTTGTTAGGCG	Patrice *et al.*, 2012
TEM	F:AAAATTCTTGAAGACG, R: TTACCAATGCTTAATCA	Jyoti *et al.*, 2008
VIM	F: GATGGTGTTTGGTCGCATA, R: CGAATGCGCAGCACCAG	Patrice *et al.*, 2012
MEC	F:AAAATCGATGGTAAAGGTTGGC, R:AGTTCTGCAGTACCGGATTTTGC	Taweeporn *et al.*, 2002
CITM	R: TTT CTC CTG AAC GTG GCT GGC, F: TGG CCA GAA CTG ACA GGC AAA	Sana *et al.*, 2015
IMP	F: GTGGTTCTTGTAAATGCTGAGG, R: CCGCCTGCTCTAATGTAAGT	Patrik *et al.*, 2016
SHV	F:ATGCGTTATATTCGCCTGT R:TGCTTTGTTATTCGGGCCAA	Nashwa *et al.*, 2017
VAN	F: GGGAAAACGACAA TTGC, R: GTACAATGCGGCCGTTA	Chimanjita *et al.*, 2017

Rifampine(RA), Azetreonum (ATM), Tetracycline (TE) and Trimethoprim-sulfamethoxaz (STX).

Molecular characterization of selected staphylococcus isolates

Total DNA materials of the selected *staphylococcus* were isolated. All selected isolates have plasmid with molecular weight less than 5000bp as shown in Fig. 1. The strains were identified by sequencing the PCR amplified 16S rDNA genes. The products of the PCR were analyzed on 1% agarose gel (Fig. 2). The sequences were easily recognized the amplified fragment of 1500bp. Assessment of 16S rRNA gene sequence for 4 selected experimental isolates with the sequences of the different type's strains from gene bank was a dominant tool to identify and classify prokaryotes (Kim *et al.*, 2014 and Zuppa, 2014).

The sequence was submitted to the BLAST database in order to find homologies with other 16S rDNA sequences. Data in figure 3 represented the similarity and accession numbers obtained after comparing the sequence of the tested strains to the submitted sequences in GeneBank. The sequence was deposited in GenBank and had the accession MK757730 for isolate 12WSZ, MK757836 for isolate 21WSZ, MK834815 for isolate31WSZ and MK757856 for isolate 37WSZ. *Staphylococcus* 12WSZ which isolated from white cheese were related to *Staphylococcus pasteuri* by 96.66 %. *Staphylococcus* 21WSZ which isolated from industrial white cheese were related to *Staphylococcus haemolyticus* by

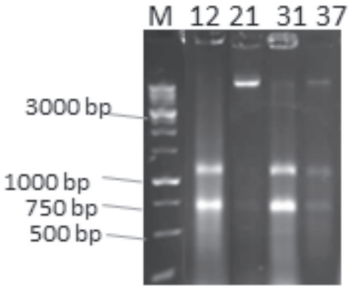

Fig. 1. Plasmid profile of selected isolates

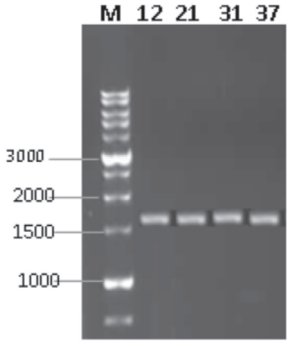

Fig. 2. PCR amplification of 16S rDNA gene (1500 bp)for the selected isolates.

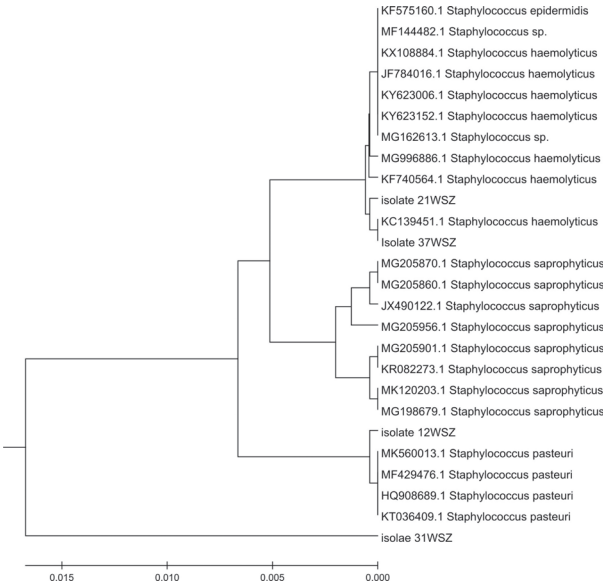

Fig. 3. Phylogenetic tree of isolate coded 12WSZ, 21WSZ, 31WSZ and 37 WSZ obtained by distance matrix analysis and phylogenetically showing the position of isolates among the selected *Staphylococcus* based on 16Sr RNA sequence comparisons.

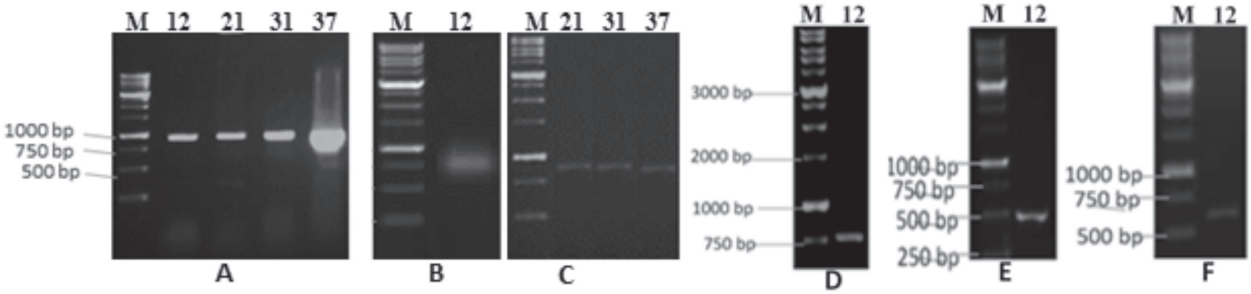

Fig. 4. PCR fragment obtained using gene specific primers A: TEM, B: KPC, C: VAN, D: VIM, E: CITM and F: SHV for the selected *Staphylococcus* isolates.

98.16%. *Staphylococcus* 31WSZ which isolated from old cheese was related to *Staphylococcus saprophyticus* by 94%. *Staphylococcus* 37WSZ which isolated from roomy cheese were related to *Staphylococcus haemolyticus* by 97%.

Detection of virulence gene in the selected staphylococcus isolates

The PCR was applied to the four selected *Staphylocoous* isolates 12, 21, 31 and 37. The collected data of absence or presence genes (KPC, TEM, VIM, MEC, CIT, IMP, SHV and VAN).The size of amplified PCR fragment was calculated using software of Gel Documentation Analysis System (Alpha Imager TM 1220) as shown in Fig. 4. The calculated size was nearer to the theoretical one according to known in publications.

DISCUSSION

Staphylococcus aureus is one of the main human pathogen that is proficient of generating a vast range of extracellular virulence elements. In a study conducted by Jeanine Allign *et al.,* (1998), they isolated and sequenced a plasmid, named pIP1714 (4,978 bp). PIP1714 was isolated from a *Staphylococcus* and consist of a 2,985-bp piece also originate in the two ring of replication, pUB110 and pBC16 present in the gram-positive bacteria. Genes for antibiotic resistance (repB and pre mob) carried on these plasmids. In our study, Results indicated that all the selected isolates of *Staphylococcus* contain plasmid <5000bp. In our study milk was free from MDR. The study by Giada Magro *et al.,* (2017) *Staphylococcus aureus* is a major agent of dairy cow in Fra-mammary infections. A greater repossession percentage was acquired by (El-Jakee *et al.,* 2008), they isolated *S. aureus* (16%) from buffalo milk and (22.7%) from cow milk but in our study no *Staphylococcus* detected in milk. In a study conducted by Rana El Feghaly *et al.* (2012) detected beta lactamase gene by PCR in 10 (9.5%) of the 105 and it is nearly similar to our study. The Study by Ali M. Badri *et al.,* 2017, the highest CTX-M of ESBL gene was representative 61%, TEM gene (16%) and SHVgene (23%). The gene of mecA related to methicillin resistance, detected in 25.7% of the *Staphylococci* isolated from cheese samples (Fontes *et al.,* 2013).

In Turkey, the genotypic description by detection of TEM gene was the supreme common gene. However an alternative study informed an occurrence of SHV higher than TEM (Capita *et al.,* 2013; Nazik *et al.,* 2011 and Zaniani *et al.,* 2011). Complement to that, the blaTEM was perceived in 91.4% of Enterobacteriaceae (Amador *et al.,* 2009). SHV and TEM is mostly produced by *K. pneumoniae* and CTX-M is mostly produced by *E. coli* (Bauernfeind *et al.,* 1992 and Paterson *et al.,* 2005). The colonization of dairy herds and subsequent contamination of raw milk by *Staphylococcus aureus*, especially those expressing a multi-drug resistance (MDR), biofilm and toxins producing ability, remains an important issue for both the dairy producer and public health. In this study, we investigated the prevalence, antimicrobial resistance, virulence, and genetic diversity of *Staphylococcus* sp. In dairy product. In the Study by Wei Wang (2018) in china, *Staphylococcus* were detect of 46.2% were positive for *S. aureus*. Resistant to penicillin (PEN) (31.3%), ciprofloxacin (18.8%) and enrofloxacin (15.6%) were the most often observed. Other study isolated bacteria with higher resistance to penicillin (73.9%), ciprofloxacin (34.8%), enrofloxacin (34.8%), tilmicosin (17.4%), and erythromycin (17.4%) (Badri *et al.,* 2017).

CONCLUSION

Fresh cheeses made with unpasteurized milk, this

may be source of contamination and the percentage differed according to region type of dairy products. Test of contamination must be measured as test of quality. Severe sterile forms must be endorsed within the fabricate, capacity and commercialization of cheese. Significantly quality ought to be down to earth fair some time recently the advance of diverse and supplementary shallow strategies for pathogen location in dairy products.

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