International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.08 pp 37-42, 2016

Molecular Screening Of Clumping Factor And Some Antibiotic Resistance Genes In Staphylococcal Isolates Obtained From Retail Pork Byproducts In Egyptian Markets

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Abstract : A total of twenty three staphylococcal isolates recovered from ready to eat local and imported pork by products were screened for presence of clumping factor A gene (*clf*A) as a mark for *Staphylococcus aureus* and three common used antimicrobial resistance genes; methicillin *(mec*A*),* erythromycin (*erm*C), and vancomycin (*van*A) using polymerase chain reaction. The results showed that 12 isolates (52.2%) have been possessed *clf*A confirmed as *S. aureus*. These twelve *S. aureus* isolates tested for the mentioned antimicrobial resistant genes, representing that two isolates carried the three genes (8.3%), five carried two genes, four carried one gene, and one isolate none. *mec*A showed the highest coexist 9 (75%) followed by *erm*C 6 (50%) then *van*A 5, (41.6%). The presence of these antimicrobial resistant genes represents a public health concern, likely, to the best of our knowledge, this is the first treatise touched the antibiotic resistant genes of isolated staphylococci from pork by products in Egyptian markets.
Keywords: *Staphylococcus aureus*, *clf*A, *mec*A, *van*A, *erm*C, pork byproducts, Egypt.

Introduction

Staphylococcus aureus is a facultative, Gram-positive grape arranged cocci, well known for its disease-causing abilities; one of the most common causes of food borne diseases due to its existence in assortments of foods[1].

One of these abilitiies is the primary attachment to a biotic surface in host tissues and synthetic surfaces coated with plasma proteins, such as fibronectin, fibrinogen, and vitronectin, this ”intercellular adhesion and biofilm “ potency is governed by cell wall-anchored (CWA) proteins such as clumping factors A which encoded by *clfA* gene[2]. Another pathogenicity character of *S. aureus* is what is called MDR organisms; which is the caplility of the organism to resist more than one antibiotic. The widish therapeutic use of antibiotics in humans and animals has shared to the increase in antibiotic resistance in pathogens[3]. Especially methicillin-resistant *S. aureus* (MRSA), which have become a serious attention regarding the public health and food safety issue. These fairs have stimulated research of MRSA in animals, and particularly pigs, in many countries. Frequent reports have attested that pig herds are an important reservoir for MRSA, with frequent detection of the same types in pork and its products[4]. Antibiotic resistance in MRSA is determined by the *mec*A gene, which encodes an altered penicillin binding protein (PBP-2a), that reduces the binding affinity for methicillin and other β-lactam antibiotics[5]. Since meat and its byproducts are important sources of human deals, it should be free of contamination and hazard[6]. Until recently, research of *S. aureus* in swines and their meat products have been heavily spotted on MRSA, with relatively little attention given to other antimicrobials as macrolides and vancomycin which have become widely used in veterinary therapeutic programs.

The objective of this study was to characterize the Staphylococcal isolates obtained from retail, local and imported pork byproducts sold in Egyptian markets with concern to their antimicrobial resistance pattern.

Materials and Methods

Bacteria: a twenty three Staphylococcal isolates previously recovered by[7] from local and imported pork byproducts .

DNA extraction: DNA extraction from the samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany) and were listed in Table (1).

PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products and 40 µl of the multiplex PCR products were loaded in each gel slot. 100 bp DNA ladder and a 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and a Generuler 100 bp ladder (Fermentas, Thermo Scientific, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed through computer software.

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension		
clfA	GCAAAATCCAGCACA ACAGGAAACGA	638	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	(8)
	CTTGATCTCCAGCCAT AATTGGTGG							
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A	310	94˚C 5 min.	94˚C 45 sec.	50˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	(9)
	CCA ATT CCA CAT TGT TTC GGT CTA A							
vanA	CATGAATAGAATAAA AGTTGCAATA	1030	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 1 min.	72˚C 10 min.	(10)
	CCCCTTTAACGCTAAT ACGATCAA							
ermC	ATCTTTGAAATCGGCT CAGG	295	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	(11)
	CAAACCCGTATTCCAC GATT							

Results and Discussion

S. aureus is a bacterium of great, ubiquitous concern due to its pathogenicity and resistance to different types of antimicrobials[12]. The ability of intercellular adhesion and biofilm formation of *S. aureus* is contributed to clumping factors A through its ability to bind fibrinogen[13], this factor is encoded by species specific part of the 23S rRNA-gene, the PCR amplification of the clumping factor (*clf*A) gene[14]. The results of 638 bp amplification of (*clf*A) gene photo [1] showed that 12 / 23 isolates (52.2%) have been positive, so confirmed as *S. aureus*.

Table (2) Screening of antimicrobial resistance genes in *S. aureus* isolates:

Sample	*clfA*	*vanA*	*mecA*	*ermC*
1	+	-	+	+
2	+	-	-	-
3	+	+	+	+
4	+	+	+	-
5	+	-	+	+
6	+	-	-	+
7	+	-	+	-
8	+	-	+	-
9	+	+	+	-
10	+	+	+	+
11	+	-	+	+
12	+	+	-	-

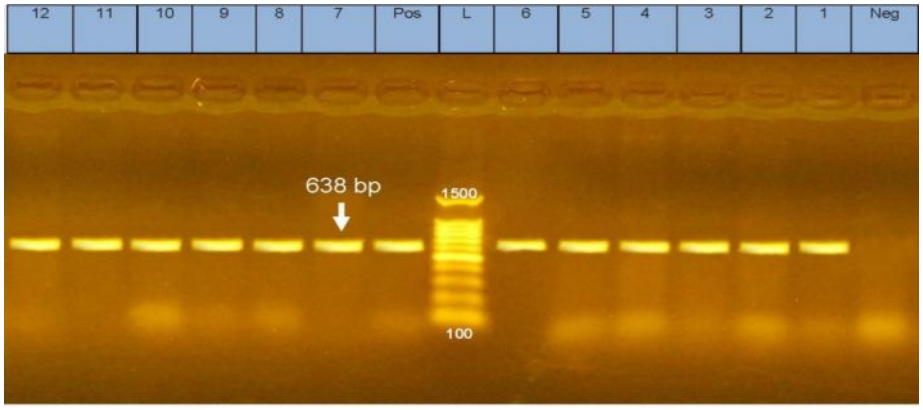

Photo (1): Detection of *clf*A in staphylococcal isolates obtained from pork byproducts
L: 100 bp DNA ladder Lanes 1-12: *staphylococci* positive *clf*A
Lane pos : positive control and Lane neg: negative control

There is rising interest in the presence of methicillin-resistant *S. aureus* (MRSA) on retail meat products. In our study, as shown in photo [2], *mec*A was detected in 9 (75%) *S. aureus* isolates and this result much closer to the antibiogram of these isolates which was carried previously [7]. *mec*A was detected in 98 out of 100 samples [15] as well as, 50% of *S. aureus* were *mec*A positive in other study, [16]. On the other hand, many researchers reported low incidences in pork; 3 /100 [(17)], 9.6% [(18)], two pork samples [6], moreover, the prevalence was 8.1% in Iowa 7.1% in Minnesota 4.6% New Jersey in retail pork products [19], and was also detected in 4% (5/135) of the retail pork [20].

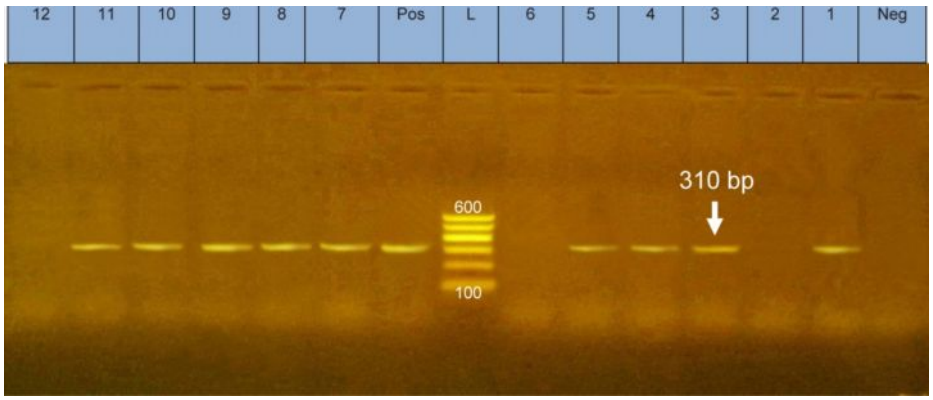

Photo (2): Detection of mecA in staphylococcal isolates obtained from pork byproducts
L: 100 bp DNA ladder 310 bp: staphylococci positive *mec*A
Lane pos : positive control and Lane neg: negative control

Results obtained in photo [3] showed that *erm*C was detected in 6 (50%), another high detection (75%) was obtained [16], moderate prevalence 35.9% [(21)], while another study found that 3.9% of staphylococci isolates carried *erm*C [22].

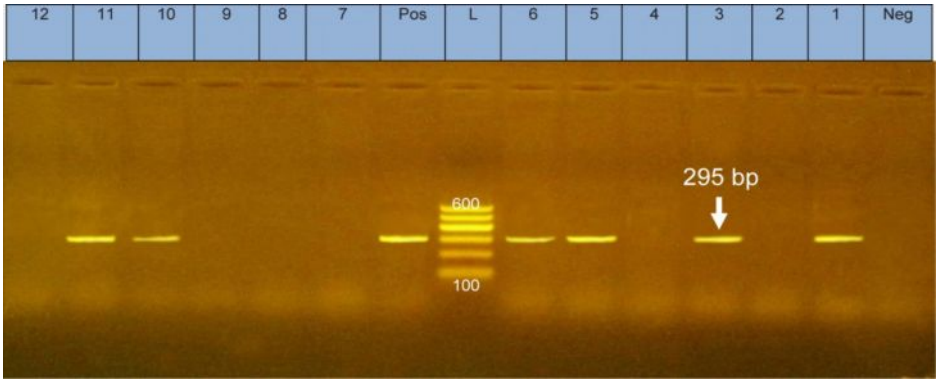

Photo (3): Detection of ermC in staphylococcal isolates obtained from pork byproducts
L: 100 bp DNA ladder 295 bp: staphylococci positive ermC
Lane pos : positive control and Lane neg: negative control

Furthermore, our data in photo [4] showed that 5 isolates, (41.6%) were positive for *van*A gene on the opposite side, the gene *van*A was not retrieved [16]. Also, the presence of *van*A gene was not detected in 154 *S. aureus* isolates recovered from 1070 food samples collected from seven cities in Turkey [23]. Wondering, it seems to be low detection of *van*A in the researches despite the high emergence of VRSA (vancomycin resistant *S. aureus* isolates) [24]. Interestingly, the results of our study indicate that more than half of the *S. aureus* isolates, 7/12 (58.3%) were MDR (multi drug resistant), which increase the threatening of the organism to human soundness.

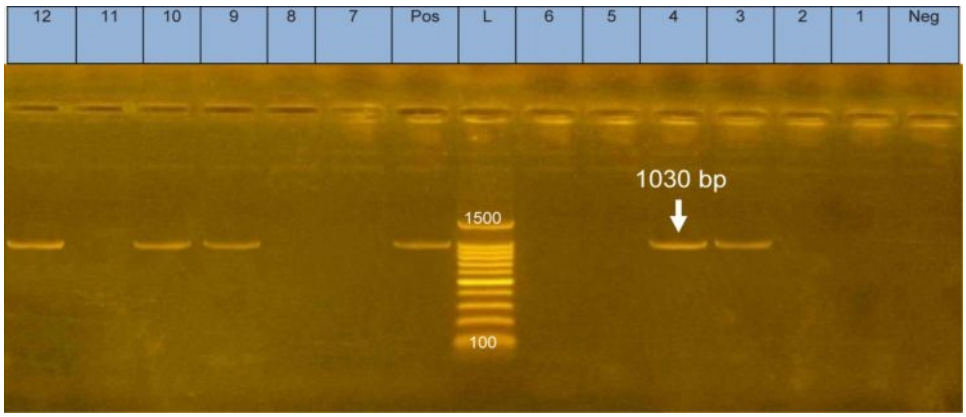

Photo (4): Detection of vanA in staphylococcal isolates obtained from pork byproducts
L: 100 bp DNA ladder 1030 bp: staphylococci positive vanA
Lane pos : positive control and Lane neg: negative control

Conclusion

Our findings showed that the local and imported pork byproducts sold in Egyptian markets could be a source of antibiotic-resistant *S. aureus* suggesting the public health hazard in the food production environment.

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