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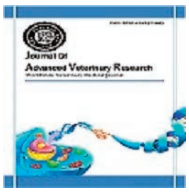

Evaluation of Some Virulence Factors of Methicillin and Vancomycin Resistant *Staphylococcus epidermidis* Isolated from Cheese and Human Samples

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ABSTRACT

Biofilm formation and enterotoxin production represent major virulence factors of *S. epidermidis* . Also, biofilm formation is greatly associated with multidrug resistance. So the objective of the present study was to expand the current knowledge regarding the importance and virulence of methicillin and vancomycin resistant *S. epidermidis* originated from dairy food, food handlers and patients in a hospital, and highlight the possible transmission through foods and food handlers. Biofilm formation was evaluated phenotypically by the tube method and microtiter plate method and genotypically through detection of *icaA* and *icaD* genes, while *S. epidermidis* isolates were investigated for potential enterotoxin production through detection of enterotoxin encoding genes (*sea*, *seb* and *sec*). Among the investigated isolates, phenotypic and genotypic biofilm formation was confirmed in 78.4 and 66.7 % of the isolates, respectively. Regarding enterotoxin encoding genes, it was found that *seb* gene was the only prevailing gene in the three categories of samples with an incidence of 27.5 %. The findings of this study illustrated the prominent role that played by food handlers in transmission of virulent *S. epidermidis* to food and subsequently patients.

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Introduction

Coagulase-negative *Staphylococci* (CoNS) constitute a major component of the normal human microfloras and have been evaluated as saprophytes. However, over the last 3 decades, the increased number of human infection cases due to CoNS has been documented representing one of the major nosocomial pathogens, with *S. epidermidis* as the serious significant species (Kloos and Bannerman, 1994; De Silva *et al.*, 2001; Vuong and Otto, 2002; Jean-Baptiste *et al.*, 2011; Becker *et al.*, 2014).

An important virulence factor associated with *S. epidermidis* is its ability to form biofilms and colonize biomaterials (Fey and Olson, 2010). Bacterial ability to form biofilms is of great importance and represents a big challenge not only for the food industry but also for the health facilities, as some strains in their sessile state may endure antimicrobial agents, making the bacterium immensely difficult to be erased (Basanisi *et al.*, 2017). In dairy industry the removal of permanently adhered cells is difficult and imposes the application of strong mechanical force or chemical interruption of the microbial adhesion through using surfactants, sanitizers or heat; even though, the probability that the irreversibly adhered cells will survive even after pasteurization is high (Schlegelova *et al.*, 2008). On the other hand, from the public health hazard point of view, biofilms are contributed to more than 80% of all infections in humans (Kot *et al.*, 2017); *S. epidermidis* is a major cause of nosocomial and implant-associated infections (Otto, 2009) due to its capacity to adhere to catheters, indwelling medical devices or to colonize different surfaces (Ferreira *et al.*, 2012; Prakash *et al.*, 2016). Biofilm formation in *S. epidermidis* , is associated with the *icaABDC* operon presence, which encodes the synthesis of the polysaccharide intercellular adhesion (PIA), which considered an important component in the process of biofilm formation and consists of a b -1,6-linked homoglycan composed of N-acetylglucosamine (Gerke *et al.*, 1998; Mack *et al.*, 2004; Liberto *et al.*, 2009; Oliveira and de Cunha, 2010). The chromosomal intercellular adhesion (*ica*) locus, consisting of the *icaA*DBC structural; and the *icaA* and *icaD* genes have been reported to play a fundamental role in biofilm consistence (Darwish and Asfour, 2013).

In the late '50s and early '70s of the twentieth century, a few reports suggested that CoNS might also produce enterotoxins in food poisoning cases (Omori and Kato, 1959; Breckinridge and Bergdoll, 1971). Enterotoxigenic CoNS including *S. epidermidis* were isolated from food and dairy products (Valle

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et al., 1990; Marín *et al*., 1992; Rodríguez *et al*., 1996; Zell *et al*., 2008; Even *et al*., 2010; Rall *et al*., 2010a) and also from cases of human clinical infections (Da Cuhna *et al*., 2007; Ataee *et al*., 2011; Vasconcelos *et al*., 2011). The presence of sequences homologous to *S. aureus* enterotoxins has been affirmed in the genomes of CoNS strains used in food processing, associated with human infection and from other environments (Weir *et al*., 2007; Madhusoodanan *et al*., 2011). Several studies have shown that some CoNS species including *S. epidermidis* possess the genes for staphylococcal enterotoxins (SE) and can produce a functional toxin (Becker *et al*., 2001; Blaiotta *et al*., 2004; Oliveira *et al*., 2011). Staphylococcal enterotoxins can be classified into five main groups (*sea*, *seb*, *sec*, *sed*, and *see*) (Rojas *et al*., 2012; De Freitas *et al*., 2013; Pinheiro *et al*., 2015).

Antibiotic resistance in CoNS, including the clinically-significant species *S. epidermidis* , represents an important health problem worldwide (Cosgrove, 2006). The prevalence of methicillin-resistant *S. epidermidis* has been increased widely and glycopeptides particularly vancomycin, which was the recommended treatment has also exhibited a failure in the treatment of some cases as a result of its resistance (Chakraborty *et al*., 2011; Morgenstern *et al*., 2016).

Biofilms formed by *S. epidermidis* decrease the metabolic activity of the bacteria and protect it from phagocytosis by effector cells (Schommer *et al*., 2011; Spiliopoulou *et al*., 2012; Hanke *et al*., 2013), also reduced the diffusion of antibiotics that makes it difficult for antibiotics to affect this type of infection (Arciola *et al*., 2010). Since, there is an association between biofilm production and the antimicrobial resistance in *S. epidermidis* (Argudín *et al*., 2015), and many other authors stated that the pathogenicity of *S. epidermidis* has ascribed to the biofilm formation beside enterotoxins production (Irlinger, 2008; Podkowik *et al*., 2013; Chaves *et al*., 2018); so, the intention of our study was to expand the knowledge regarding the virulence potential of methicillin and vancomycin resistant *S. epidermidis* isolates from dairy foods, food handlers and patients in the hospital through: a) Detection the ability of these strains to phenotypic formation of biofilm; b) Molecular detection of biofilm encoding genes (*icaA* and *icaD*) . c) Molecular detection of staphylococcal enterotoxins genes *sea*, *seb*, and *sec*.

Materials and methods

S. epidermidis isolates

A total of 51 *S. epidermidis* isolates showed resistance to both methicillin and vancomycin antibiotics (MR/VR- *S. epidermidis*) were obtained in a study conducted by El-Zamkan *et al.* (2019) at a hospital in Qena City, Egypt, on 210 samples that examined for detection of methicillin and/or vancomycin resistant CoNS including 90 dairy food samples which offered to the patients in the hospital (soft cheese, processed cheese, and yoghurt; 30 samples for each), 60 nasal and hand swabs from food handlers working in the hospital (30 for each) and 60 nasal and diarrheal swabs from patients admitted to the hospital and suffered from diarrhea (30 for each). In this study, the isolates were evaluated for their enterotoxigenecity and ability to form biofilm representing the most important *S. epidermidis* hazardous virulence factors.

Ethical approval is not required in case of food samples while oral consent was obtained from each participant patient.

Isolation and detection of methicillin and vancomycin resistant S. epidermidis

Human samples were collected in sterile plastic containers with sterile saline solution (0.9% NaCl) by using sterile swabs and transported to the laboratory, then one ml of each human sample was inoculated in 9 ml sterile buffered peptone water (Oxoid CM0509, Basingstoke, Hampshire, England), while 10 gm of each food sample was homogenized in 90 ml sterile buffered peptone water (Oxoid, CM0509). Buffered peptone water was incubated overnight at 37 °C. Then, a loopful of each sample was streaked on Mannitol Salt Agar (Merck, Germany) and incubated aerobically at 37 °C for 24 h for growth; pink colonies were considered as CoNS. Identification of CoNS species was done according to Kloos and Schleifer (1975) and Bannerman (2003). Resistance to methicillin and vancomycin was detected phenotypically according to the guidelines of the National Reference Centre for Antimicrobial Susceptibility and internationally recognized standards of the Clinical and Laboratory Standards Institute (CLSI, 2014) on Muller Hinton agar (Merck, Germany) using the diffusion disk method. Later, the isolates that showed phenotypic antibiotic resistance were submitted to PCR to confirm presence of *mecA* gene F (5'- GTA GAA ATG ACT GAA CGT CCG ATA A-3') and R (5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3') (McClure *et al*., 2006), and *vanA* gene F (5'- GGGAAAACG ACAATTGC -3') and R (5'- GTACAAT-GCGGCCGTTA -3') (Depardieu *et al*., 2004).

Biofilm Formation tests

Tube method (TM)

Biofilm production was examined by the tube adherence test proposed by Christensen *et al*. (1982) in which 10 mL of Trypticase soy broth with 1% glucose was inoculated with a loopfull of the overnight culture on nutrient agar individually. Broths were incubated at 37 °C for 24 h. The cultures were decanted and tubes were washed with phosphate buffer saline (pH 7.3). The tubes were dried and stained with 0.1% crystal violet. Excess stain was washed with deionized water. Tubes were dried in an inverted position and observed for biofilm formation. Biofilm Production was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation.

Microtiter plate (MTP) method

The technique was done as described by Stepanović *et al*. (2000). Each MR/VR- *S. epidermidis* isolate was grown on Tryptic-Soy Agar with 1% glucose at 37 °C overnight, as the growth medium. Then a dilution 1:100 was prepared in Tryptic soy broth (TSB) medium supplemented with glucose (1%) followed by filling three wells of a sterile 96- well flat-bottomed polystyrene tissue culture plate with a lid with 200 µL of aliquots of the diluted culture; negative control wells contained uninoculated sterile broth. The plates were covered and incubated for 24 h at 37 °C. The contents of each well were gently aspirated, and the wells were washed three times with 250 µL of phosphate-buffered saline (PBS). The remaining adhered bacterial cells were fixed with 200 µL of 99% methanol for 15 min., then plates were air-dried after methanol removal, After that wells were stained with 0.2 mL of 0.2% crystal violet (CV) for five min. Then, wells were rinsed twice with distilled water and dried thoroughly. For the quantification of biofilm growth, the adherent cells were resolubilized with glacial acetic acid (33%). The OD of the resulting solutions in each well was measured at 570 nm with a microplate reader. Based on the OD of the bacterial film, all strains were classified into the following categories: "OD≤ODc: non–adherent, ODc<OD≤2×ODc: weakly adherent, 2×ODc<OD≤4×ODc: moderately adherent, 4×ODc<OD: strongly adherent". All

tests were carried out in triplicates and the results were averaged. Isolates were considered biofilm-positive when they have an OD570 nm > 0.2. Each isolate was tested in triplicate and the results were averaged.

Molecular detection of enterotoxins and biofilm associated genes

Biofilm encoding genes and enterotoxin genes detected in this study and their amplification conditions are listed in Table 1. Primers were supplied from Metabion (Germany).

DNA extraction

DNA extraction 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 °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.

Biofilm production and analysis of *icaA/D* genes

Primers were utilized in a 25 µL reaction containing 12.5 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), one µL of each primer of 20 pmol concentrations, 4.5 µL of water, and 6 µL of DNA template. The reaction was performed in an Applied Biosystem 2720 thermal cycler.

Enterotoxins detection

Primers of *sea*, *seb*, and *sec* genes were utilized in a 50 µL reaction containing 25 µL of EmeraldAmp Max PCR Master Mix (Takara, Japan), one µL of each primer of 20 pmol concentration, 8 µL of water, and 7 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. Gelpilot 100 bp and 100 bp plus DNA ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Statistical analysis

Bacterial count and OD variables, significant factors ($P \leq 0.05$) and agglomerative hierarchical clustering (ACH) performed using XLSTAT 2016 software for Microsoft Excel® (Microsoft®, WA, USA).

Results

Of the all 51 methicillin and vancomycin resistant *S. epidermidis* isolates obtained from cheese, food handlers and pa-

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

	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	References
			Primary denaturation	Secondary denaturation	Annealing	Extension		
sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	94˚C 5 min.	94˚C 30 sec.	57 ˚C 40 sec.	72˚C 40 sec	72˚C 10 min.	Mehrotra *et al.* (2000)
seb	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164						
sec	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451						
icaA	CCT AAC TAA CGA AAG GTA G AAG ATA TAG CGATAA GTG C	1315	94˚C 5 min	94˚C 30 sec	49˚C 1 min.	72˚C 1 min.	72˚C 10 min	Ciftci *et al.* (2009)
icaD	AAA CGTAAG AGA GGT GG GGC AAT ATG ATC AAGATA	381	94˚C 5 min	94˚C 30 sec	49˚C 30 sec.	72˚C 30 sec.	72˚C 10 min	

Table 2. Prevalence of *icaA* and *icaD* genes in *S. epidermidis* isolates and phenotypic biofilm production evaluated by microtitre plate method (MPT) and tube method.

Source of isolates (No.)		Biofilm genes						Biofilm Production									
								MTP						Tube Method			
		icaA		*icaD*		*icaA* and *icaD*		SA[a]		MA[b]		WA[c]		+ve		-ve	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Dairy Food	Cheese (n.=13)	3	23.1	10	76.9	3	23.1	5	38.5	2	15.4	3	23.1	7	53.8	6	46.2
Food Handlers	Nasal Swab (n.= 6)	1	16.7	4	66.7	1	16.7	2	33.3	2	33.3	0	0.0	4	66.7	2	33.3
	Hand Swab (n.= 7)	1	14.3	5	71.4	1	14.3	2	33.3	2	33.3	1	14.3	4	57.1	3	42.9
Total	13	2	15.4	9	69.2	2	15.4	4	30.8	4	30.8	1	7.7	8	61.5	5	38.5
Hospitalized Patients	Nasal Swab (n.= 16)	5	31.3	7	43.8	3	18.8	4	25.0	2	12.5	8	50.0	8	50.0	8	50.0
	Diarrheal Swab (n.= 9)	2	22.2	6	66.7	2	22.2	3	33.3	2	22.2	2	22.2	4	44.4	5	55.6
Total	25	7	28.0	13	52.0	5	20.0	7	28.0	4	16.0	10	40.0	12	48.0	13	52.0
Overall Total	51	12	23.5	32	62.7	10	19.6	16	31.4	10	19.6	14	27.5	27	52.9	24	47.1

a=strong adherent; b= moderate adherent; c= weakly adherent

tients in a hospital in Qena Governorate, 23.5, 62.7 and 19.6% were positive for the presence of *icaA*, *icaD* and both *icaA* & *icaD* genes, respectively (Table 2 and Figs. 1 and 2). Out of the 51 isolates, 27 versus 40 isolates showed biofilm-forming phenotypes using tube and microtiter plate (MPT) methods, respectively; and the later ranged from weak to strong adherent (Table 4). The frequency distribution of genes and biofilm phenotype detected is shown in Fig. 3. There were no statistically significant differences between the presence of *icaA* and/or *icaD* gene and biofilm formation using MTP method (P> 0.05), while the accompanying presence of both *icaA* and *icaD* was significantly associated with biofilm phenotype (P< 0.05).

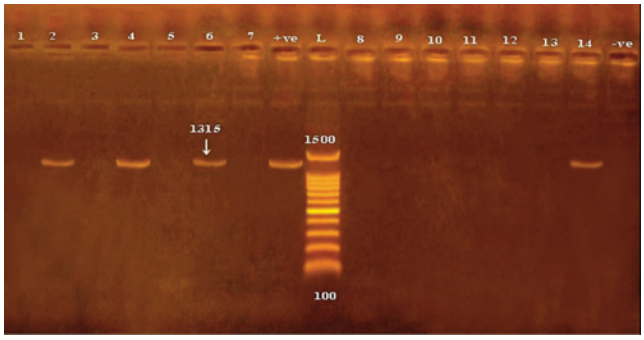

Fig. 1. Detection of *icaA* gene in methicillin and vancomycin resistant *S. epidermidis* isolates obtained from cheese and human samples. L: Ladder, +ve: positive control, -ve: negative control.

According to data showed in Table 3, of all the investigated isolates 27.5% (14/51) were harboring *seb* gene, including 30.8% (4/13) of each dairy food and food handlers' isolates and 24% (6/25) of hospitalized patients, while *sea* gene was found in only one *S. epidermidis* isolate that originated from cheese sample (Fig. 4).

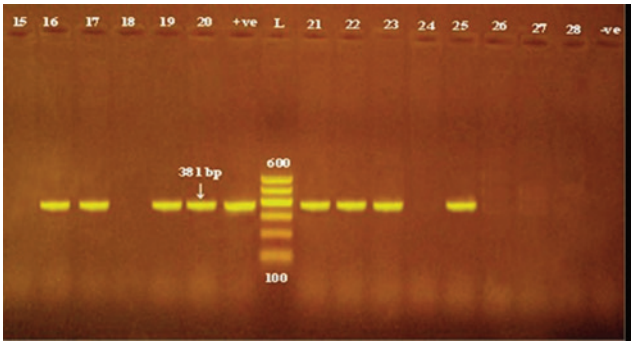

Fig. 2. Detection of *icaD* gene in methicillin and vancomycin resistant *S. epidermidis* isolates obtained from cheese and human samples. L: Ladder, +ve: positive control, -ve: negative control.

Discussion

There is a limited data regarding phenotypic and genotypic biofilm production patterns in *S. epidermidis* isolated from dairy food. Additionally, the incidences of strains that possessed *icaA* and/or *icaD* genes and showed a phenotypic biofilm formation in the current work should be interpreted

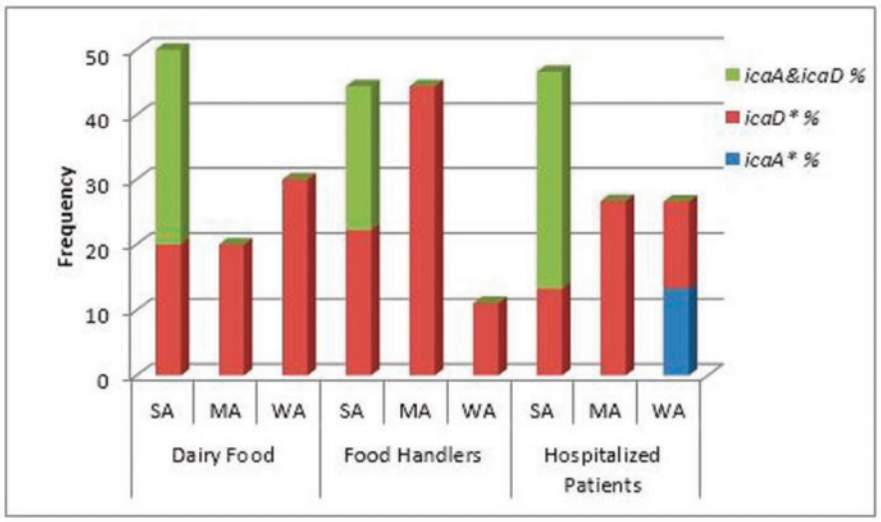

Fig. 3. Frequency distribution of biofilm genes in relation to phenotypic biofilm formation (*SA=strong adherent; MA= moderate adherent; WA= weakly adherent; *icaD**=icaD* gene only; *icaA*= *icaA* gene only).

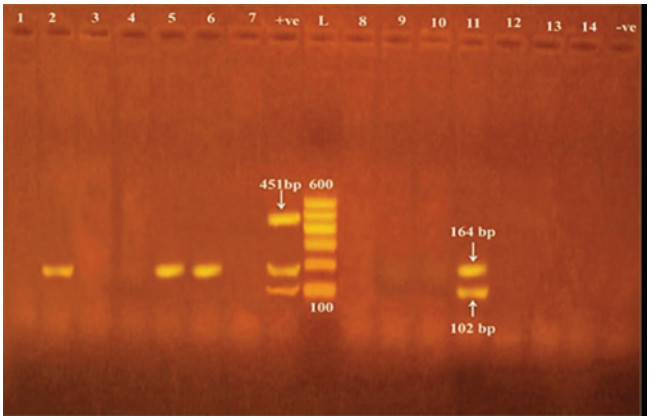

Fig. 4. Detection of *sea*, *seb* and *sec* genes encoding staphylococcal enterotoxin production in methicillin and vancomycin resistant *S. epidermidis* isolates obtained from cheese and human samples. L: Ladder, +ve: positive control, -ve: negative control

Table 3. Prevalence of enterotoxin genes in *S. epidermidis* isolates

Source of samples (No.)		*sea*		*seb*		*sec*	
		No.	%	No.	%	No.	%
Dairy foods	Cheese (n.=13)	1	7.7	4	30.8	0	0.0
Food handlers	Nasal swab (n.=6)	0	0.0	2	33.3	0	0.0
	Hand swab (n.=7)	0	0.0	2	28.6	0	0.0
Total	13	0	0.0	4	30.8	0	0.0
Hospitalized patients	Nasal swab (n.=16)	0	0.0	2	12.5	0	0.0
	Diarrheal swab (n.=9)	0	0.0	4	44.4	0	0.0
Total	25	0	0.0	6	24.0	0	0.0
Over all total	51	1	1.9	14	27.5	0	0.0

with caution because the study was confined to a certain group of small *S. epidermidis* isolates number, which all shared resistance to both methicillin and vancomycin, which may be the reason for reporting such higher results in cheese than Schlegelová *et al.* (2008; Chajęcka-Wierzchowska *et al.* (2019), and in food handlers than Udo *et al.* (2009), while in patients it was higher than Ninin *et al.* (2006); Gad *et al.* (2009); Saising *et al.* (2012); Ebrahimi *et al.* (2014) and Kord *et al.* (2018). However, in human samples, many other authors like Smith *et al.* (2008) and Piechota *et al.* (2018) recorded higher results as *S. epidermidis* is more abundant in human including patients and healthy persons (Schlegelova *et al.*, 2008; Jaglic *et al.* 2010).

From the presented results, it is clear that *icaD* gene was the most detected biofilm gene in the isolates either separately or in combination with *icaA*. In the same way, de Mello *et al.* (2014) and Chajęcka-Wierzchowska *et al.* (2019) detected *icaD* gene in a higher proportion than *icaA* gene in *S. epidermidis* isolated from cheese and food handlers, respectively. Unlike our results, *icaA* was the most detected gene in *S. epidermidis* isolated by Pinheiro *et al.* (2016) and by Hasanvand *et al.* (2019), while no mentioned difference between the incidence of *icaA* and *icaD* genes in *S. epidermidis* collected from milk samples was found by Abbondio *et al.* (2019).

De Silva *et al.* (2002) described an association between the *ica* operon and quantitative biofilm formation. In the current study, the emphasis is on the importance of biofilm formation in the pathogenesis of MR/VR- *S. epidermidis* , particularly the role that may be played by *icaA* and/or *icaD* genes in the phenotype of the formed biofilm. In the current study, most isolates contained *icaD* gene produced biofilm ranged from weak to strong; while the concomitant presence of both *icaA* and *icaD* was significantly associated with biofilm phenotype ($P < 0.05$) as all strains that co-expressed *icaA* with *icaD* were strong biofilm formers. Similarly, Cafiso *et al.* (2004); Pinheiro *et al.* (2016) and Chajęcka-Wierzchowska *et al.* (2019) found that the presence of *icaA* and *icaD* was significantly associated with the biofilm formation results. Unlike these findings, Ninin *et al.* (2006) could not find such a relation.

Also, it was found that only two isolates obtained from nasal swabs of patients were found to have only *icaA* gene and theses isolates associated with weak biofilm phenotype. This genotypic, phenotypic correlation was explained in a study conducted by Gerke *et al.* (1998) where they found that presence of *icaA* alone revealed a weak N-acetylglucosaminyl transferase activity, while its full activity is only achieved when *icaD* is co-transcribed with *icaA*; the PIA consists of a b-1,6-linked homoglycan composed of N-acetylglucosamine, which is a substrate for the glycosyltransferase reaction in PIA biosynthesis.

Results illustrated in this study revealed that, neither *icaA* nor *icaD* could be detected in non-biofilm formers which originated from cheese and food handlers and this disagree with Los *et al.* (2010); Abu Taleb *et al.* (2012); Abbondio *et al.* (2019); Chajęcka-Wierzchowska *et al.* (2019). On the other hand, in this study six isolates obtained from patients as well as isolates obtained by Bartoszewicz *et al.* (2004); Qin *et al.* (2007); Schommer *et al.* (2011); Darwish and Asfour (2013); Lira *et al.* (2016) and Pinheiro *et al.* (2016) showed the ability to produce biofilm while they were *ica* negative strains; contrarily, Rohde *et al.* (2004) and Salgueiro *et al.* (2017) reported that PCR positivity for *icaA*/*icaD* genes, can also be found in non-biofilm producers. This conflict between studies could be contributed to many theories a): Biofilm formation is largely a strain-specific trait (Mack *et al.*, 2004); b): Regulation of the *ica* operon appears to be very complex. Production of PIA is certainly subjected to on-off switching and may be involved in *S. epidermidis* phase-variation that might improve bacterial survival and growth under changing environmental conditions in vivo (Ninin *et al.*, 2006).

The microtitre plate method is considered as the golden standard method for biofilm detection as it quantified the formed biofilm in 40/51 *S. epidermidis* , while tube method identified 24/51 isolates as a non-biofilm producer which may be due to the difficulty to differentiate between weak and non-biofilm because the interpretation of the results of tube method varies according to each person observation, also differences in biofilm formation between phenotypic methods may be due to the effect of different culture media, pH, temperature, and osmotic pressure (Deka, 2014).

The high incidences of phenotypic and genotypic biofilm *S. epidermidis* producer in this study as well as other studies performed by De Silva *et al.* (2002); Cafiso *et al.* (2004); Prasad *et al.* (2012); Sahal and Bilkay (2014); Argudín *et al.* (2015) and Chajęcka-Wierzchowska *et al.* (2019) confirmed that biofilm formation and antibiotic resistance are considered to be one of the main virulence factors and are associated with each other and are regarded as important markers that differentiate commensal and pathogenic strains; also strains that are stronger biofilm formers are often multidrug resistant.

Although CoNS safety hazards associated with the presence of methicillin and vancomycin antibiotic resistance and the mutual role that played by food and food handlers in its transmission have been illustrated in a study performed by El-Zamkan *et al.* (2019), it was important to investigate some CoNS isolates (*S. epidermidis*) found in that study for their positivity for enterotoxin genes, which have been reported for its existence and for being frequent causes of food poisoning outbreaks that initiated through consumption dairy food (Rosec *et al.*, 1997; Carmo *et al.*, 2002; Veras *et al.*, 2003; Park *et al.*, 2011).

The molecular method can be considered as the routine analytical method for SE detection. Although, staphylococcal enterotoxin SEA is the most common cause of staphylococcal food poisoning worldwide, but the involvement of other staphylococcal enterotoxins has been also demonstrated (Argudín *et al.*, 2010). The results of this study revealed that *seb* gene was the most prevailing gene (30.8%) in *S. epidermidis* isolates originated from cheese samples followed by *sea* gene (7.7%), while *sec* gene could not be located in these isolates. Results obtained by Even *et al.* (2010) pointed out that 76%

Table 4. Virulence profile of *S. epidermidis* strains isolated from cheese, food handlers and hospitalized patients.

Strain No.		*icaA, icaD*	*Sea, seb ,sec*	Biofilm production		Origin
				Tube Method	MTP	
1	*S. epidermidis*	*icaD*	-	+ve	Strong	White cheese
2	*S. epidermidis*	*icaA, icaD*	*seb*	+ve	Strong	
3	*S. epidermidis*	*icaD*	-	-ve	Weak	
4	*S. epidermidis*	*icaA, icaD*	-	+ve	Strong	
5	*S. epidermidis*	-	*seb*	-ve	Non-adherent	
6	*S. epidermidis*	*icaA, icaD*	*seb*	+ve	Strong	
7	*S. epidermidis*	-	-	-ve	Non-adherent	
8	*S. epidermidis*	*icaD*	-	-ve	Weak	
9	*S. epidermidis*	-	-	-ve	Non-adherent	
10	*S. epidermidis*	*icaD*	-	+ve	Moderate	
11	*S. epidermidis*	*icaD*	*sea, seb*	+ve	Strong	
12	*S. epidermidis*	*icaD*	-	-ve	Weak	
13	*S. epidermidis*	*icaD*	-	+ve	Moderate	Processed cheese
14	*S. epidermidis*	*icaA, icaD*	-	+ve	Strong	Food Handlers (Nasal swab)
15	*S. epidermidis*	-	*seb*	-ve	Non-adherent	
16	*S. epidermidis*	*icaD*	*seb*	+ve	Moderate	
17	*S. epidermidis*	*icaD*	-	+ve	Moderate	
18	*S. epidermidis*	-	-	-ve	Non-adherent	
19	*S. epidermidis*	*icaD*	-	+ve	Strong	
20	*S. epidermidis*	*icaD*	-	-ve	Moderate	Food Handlers (Hand Swab)
21	*S. epidermidis*	*icaA, icaD*	*seb*	+ve	Strong	
22	*S. epidermidis*	*icaD*	-	+ve	Moderate	
23	*S. epidermidis*	*icaD*	-	+ve	Weak	
24	*S. epidermidis*	-	-	-ve	Non-adherent	
25	*S. epidermidis*	*icaD*	-	+ve	Strong	
26	*S. epidermidis*	-	*seb*	-ve	Non-adherent	
27	*S. epidermidis*	-	-	+ve	Weak	Hospitalized patients (Nasal swabs)
28	*S. epidermidis*	-	-	-ve	Non-adherent	
29	*S. epidermidis*	*icaA, icaD*	-	+ve	Strong	
30	*S. epidermidis*	-	-	-ve	Weak	
31	*S. epidermidis*	-	-	-ve	Weak	
32	*S. epidermidis*	*icaA, icaD*	-	+ve	Strong	
33	*S. epidermidis*	*icaD*	-	+ve	Weak	
34	*S. epidermidis*	*icaD*	*seb*	-ve	Strong	
35	*S. epidermidis*	*icaA*	-	+ve	Weak	
36	*S. epidermidis*	-	-	-ve	Non-adherent	
37	*S. epidermidis*	*icaA*	-	+ve	Weak	
38	*S. epidermidis*	*icaA, icaD*	-	+ve	Strong	
39	*S. epidermidis*	-	*seb*	-ve	Weak	
40	*S. epidermidis*	-	-	-ve	Weak	
41	*S. epidermidis*	*icaD*	-	+ve	Moderate	
42	*S. epidermidis*	*icaD*	-	-ve	Moderate	
43	*S. epidermidis*	*icaA, icaD*	*seb*	+ve	Strong	Hospitalized patients (Diarrheal Swab)
44	*S. epidermidis*	*icaD*	*seb*	-ve	Strong	
45	*S. epidermidis*	-	-	-ve	Non-adherent	
46	*S. epidermidis*	-	*seb*	+ve	Weak	
47	*S. epidermidis*	*icaD*	-	-ve	Moderate	
48	*S. epidermidis*	-	-	-ve	Non-adherent	
49	*S. epidermidis*	*icaA, icaD*	-	+ve	Strong	
50	*S. epidermidis*	*icaD*	-	+ve	Weak	
51	*S. epidermidis*	*icaD*	*seb*	-ve	Moderate	

of the strains carrying genes encoding staphylococcal toxin belonged to *S. epidermidis* species isolated from fermented food including cheese, while, Guimarães *et al.* (2013) found that 63.2% *S. epidermidis* strains isolated from milk showed enterotoxin-encoding genes and *sea* and *seb* were the most dominating, while, Rodrigues *et al.* (2017) detected enterotoxin genes in *S. epidermidis* isolated from 3 different processing plants of fresh cheese but none of *sea*, *seb* or *sec* was amongst them. Contrarily, Rall *et al.* (2010a), Siqueira *et al.* (2017) and Abbondio *et al.* (2019) reported that none of the enterotoxin genes (*sea*, *seb*, *sec*) could be detected in *S. epidermidis* isolated from milk.

Also, the results of this study revealed that *seb* gene only could be detected in 30.8 and 24% of *S. epidermidis* isolates obtained from food handlers and patients, respectively. This obtained result comes in parallel to data recorded by Ahanotu *et al.* (2006) and Sospedra *et al.* (2012), which proved that *seb* gene is the most common gene in coagulase negative *Staphylococci* identified from foods and food handlers. The gene was detected in closely related result in multidrug resistant *S. epi-*

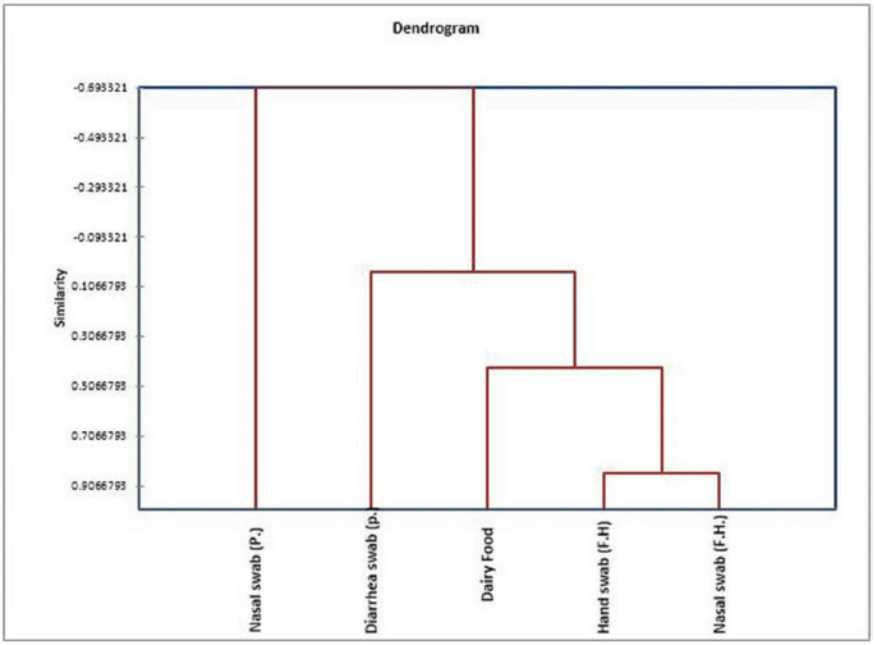

Fig. 5. Dendrogram showing the relatedness between MR/VR *S. epidermidis* isolates. The dendrogam is based on the presence/absence of *seb* and biofilm-forming genes (*icaA* and *icaD*)

dermidis isolated from food handlers in a hospital as 35% by Francisco and Moreno (2018); also in a study achieved by Udo *et al.* (2009) 38% of *S. epidermidis* isolates produced different SEs with the majority for *seb* gene. In contrary to this result, a lower incidence of *seb* gene (17.2%) could be detected by Rall *et al.* (2010b). The enterotoxin production by *S. epidermidis* was also studied by several authors as Rapini *et al.* (2005) who observed that *S. epidermidis* isolated from food handlers produced *sea* and sed in 96.4%. A smaller prevalence of CoNS enterotoxin-encoding genes was observed by Crass and Bergdoll (2007), as 16.5% of CoNS strains obtained from food handlers. While the higher prevalence of *seb* gene producing *S. epidermidis* strains isolated from food handlers was observed by Santos (2003) as 63.2%. On the other hand, Çepoğlu *et al.*, (2010) couldn't detect *seb* gene in *S. epidermidis* isolates from food handlers and detected only see gene.

Regarding hospitalized patients, closely related results to ours were obtained by Pinheiro *et al.* (2015) who recorded that 30% of *S. epidermidis* isolates obtained from patients contained *seb* gene. The production of the classical enterotoxins *sea*, *seb*, and *sec* by clinical isolates of multidrug resistant *S. epidermidis* from hospitalized patients has been also described by Cunha *et al.* (2007); Barretti *et al.* (2009); Rojas *et al.* (2012) and Vasconcelos *et al.* (2011). The differences between studies may be related to variation in the isolates studied, including the number, nature and geographic origin of the strains.

Clustering the isolates according to its detected virulence factors (The presence or absence of *icaA*, *icaD* and *seb* genes) illuminated the close relationship between samples of human handlers and dairy foods followed by their closer relationship with diarrheal samples, while the nasal swabs of patients were located in a separate arm (Fig. 5). This cluster highlights the mutual role played by food handlers in transmitting virulent *S. epidermidis* to food through cross contamination and the role of these strains in initiating foodborne diseases through food consumption. This was also observed by Doulgeraki *et al.* (2017) who stated that methicillin resistant *Staphylococci* could produce biofilms by contamination from human handlers rather than from food itself; yet in this study food handlers cannot be considered as standard community isolates as the hospital personnel can act as a reservoir or as a vector for clinical *S. epidermidis* strains (Milisavljevic *et al.*, 2005).

Conclusion

The data obtained in this study pointed out the linkage between the antibiotic resistance and biofilm formation by *S. epidermidis* and also the relation of *icaA* and *icaD* genes expression and biofilm phenotype. Additionally, the occurrence of staphylococcal enterotoxin genes in isolates of this study highlights their potentiality to cause food poisoning. Hence, Biofilm together with enterotoxin production are the most important virulence factors of *S. epidermidis* . All the above findings are dominated by the conspicuous role played by food handlers in the transmission of the virulent *S. epidermidis* to food or patients.

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Conflict of interest

The authors declare that they have no conflict of interest.

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