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ORIGINAL ARTICLE

Prevalence and phylogenetic relationship among methicillin- and vancomycin-resistant *Staphylococci* isolated from hospital's dairy food, food handlers, and patients

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ABSTRACT

Objective: The aim of the present work was to investigate the mutual role that may be played by the served dairy food and food handlers in the transmission of methicillin- and vancomycin-resistant *Staphylococcus aureus* and coagulase-negative *Staphylococci* to patients who were hospitalized in Qena City, Egypt.

Materials and Methods: A total of 210 samples including 90 dairy food samples which offered to the patients in the hospital, 60 nasal and hand swabs from food handlers working in the hospital, and 60 nasal and diarrheal swabs from patients suffering from diarrhea were investigated for the presence of coagulase-positive *S. aureus* and coagulase-negative *Staphylococci*, then isolates were screened for methicillin and vancomycin resistance phenotypically and genotypically. *16s rRNA* gene sequencing was employed to construct the neighbor-joining tree.

Results: Unlike food samples, both coagulase-positive *S. aureus* and coagulase-negative *Staphylococci* occurred in human samples. Methicillin- and vancomycin-resistant coagulase-negative *Staphylococci* could be detected in 41.7% & 20.8%, 68% & 31.9%, and 81.3% & 55.2% of isolates obtained from dairy food, food handlers, and patients' samples, respectively. Whereas 81% & 64.3%, and 75.4% & 38.6% of coagulase-positive *S. aureus* obtained from food handlers and patients' samples exhibited resistance to methicillin and vancomycin, respectively. Phenotypic resistance was confirmed molecularly through detection of *mecA* and *vanA* genes.

Conclusion: A significant role can be played by food and food handlers in the transmission of methicillin- and vancomycin-resistant *Staphylococci* to patients, which has been proved in this study through the close phylogenetic relation between *S. epidermidis* isolated from food, food handlers, and patients' diarrheal samples.

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Dairy food; food handlers; methicillin & vancomycin resistance; patients; *Staphylococci*

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Introduction

Staphylococci are among the dominant groups of saprophytic bacteria; however, lately, they are globally famous for being opportunistic pathogens and prime causes of community-associated and hospital-acquired infections in humans and animals [1,2]. The coagulase-positive *Staphylococcus aureus* (CoPSA) is a leading human pathogen accountable for an enormous range of diseases [3,4], and exhibits the highest pathogenic potential among *Staphylococci*, while, the coagulase-negative *Staphylococci* (CoNS) were evident to be saprophytic than pathogenic to a great extent [5]. But lately, CoNS became one of the leading etiologies that accountable for nosocomial bloodstream infection extending from mild skin and soft tissue infections to life-threatening diseases like septicemia, endocarditis, and necrotizing pneumonia, in humans [6]. In terms of food safety, although CoPSA is used to be famous for being the main causative agent of foodborne illness due to the production of staphylococcal enterotoxins (SEs) [7], the enterotoxigenic potential of CoNS species in foodborne illness has also been recently realized as a result of detection of SEs genes in CoNS isolates by several authors like Nunes et al. [8] and Veras et al. [9].

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Antimicrobial resistance is an important health problem worldwide [10] and it is an important factor in defining the pathogenicity of *Staphylococci*. The voluminous therapeutic use of antimicrobials, substandard concentrations of antibiotics in the patients, and implementation of sub-therapeutic doses of antibiotics to promote growth in food animals motivated the resistance in both animal and human pathogens [11,12]. Methicillin and vancomycin resistance remain the major antimicrobial resistance phenotype of concern. Most research studies concerning antibiotic resistance of *Staphylococci* focus on methicillin-resistant CoPSA (MRSA) due to the fact that it is distributed worldwide and constitutes an immense worry in human health because of its complicated epidemiology and its ability to develop novel antibiotic resistance mechanisms [13,14], whereas CoNS species did not get enough attention, although it generally carries enormous variety of *SCCmec* elements, in which the *mec* gene that encoding the penicillin-binding protein 2α (PBP2α) and causing resistance, is embedded; and are believed to be the apparent reservoir of the various types of *mecA* gene in MRSA [15–17]. Methicillin-resistant CoNS (MRCoNS) recently are gaining attention as the frequently detected causative agent of human and animal infections [6,18]. The glycopeptide antibiotic vancomycin is considered the effective antimicrobial for infections resulted from methicillin-resistant *Staphylococci* and diffuse utilization of vancomycin has induced the evolution of strains with reduced sensitivity to vancomycin [19].

Food industry could perform a role in the appearance and spreading of antibiotic resistance through transmission of resistant bacteria to humans through food [20] and vice versa through food handlers which are a potential cause of infections by many foodborne pathogens since many diseases are communicable and caused originally by microorganisms carried into their bodies [21]. So this work was aimed to assess the potential risk of methicillin- and vancomycin-resistant *Staphylococci* transmission through dairy foods and food handlers at a hospital in Qena City, Egypt.

Materials and Methods

Ethical approval

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

Study design

The current study was conducted to study the prevalence of CoPSA and CoNS in different dairy food offered, food handlers as well as patients in a hospital in Qena City, Egypt.

Samples collection

Ninety samples were collected from foods which offered to the patients in the hospital including soft white cheese, processed cheese, and yogurt (30 samples for each). Also, nasal and hand swabs were collected from 30 food handlers working in the hospital using sterile swabs moistured with sterile saline solution (0.9% NaCl). A swab was used to swab regions in between fingers and the palms of right and left hands; another swab was scrubbed over the frontal nares of both nostrils and rotated for 5 sec. At the same time, 60 nasal and diarrheal swabs (30 for each) were collected from patients who entered the hospital and suffering from diarrhea. Swabs were transported to the laboratory in sterile plastic containers with 2 ml sterile saline solution (0.9% NaCl).

Isolation and identification of Staphylococcus species

Nine ml sterile buffered peptone water (Oxoid CM0509, Basingstoke, Hampshire, England) was inoculated with 1 ml of each human sample, while 10 gm of each food sample were blended with 90 ml sterile buffered peptone water (Oxoid, CM0509) and then incubated overnight at 37°C. Then, 0.1 ml of each sample was streaked on Mannitol Salt Agar (Oxoid, CM0085) which incubated at 37°C for 24–48 h for growth; yellow colonies were considered CoPSA, while pink colonies were considered CoNS. Conventional microbiology procedure was performed following instructions to the Bergey's manual of systematic bacteriology [22], and identification of CoNS species was done according to Kloos and Schleifer [5] and Bannerman [23].

Phenotypic detection of antibiotic resistance

Resistance to antibiotics was examined in accordance with the directions of the National Reference Centre for Antimicrobial Susceptibility and internationally known standards of the Clinical and Laboratory Standards Institute [24]. Determinations were proceeded using the diffusion disk method; where three colonies of each contaminated sample with *Staphylococci* were cultured on Müeller–Hinton agar (Oxoid, CM0337) with Oxacillin (1 µg) (Oxoid, CT0159B) and Vancomycin (30 µg) (Oxoid, CT0058B) disks; plates were incubated for 24 h at 37°C.

Molecular detection of antibiotic resistance

The isolates that showed antibiotic resistance were submitted to PCR targeting *clfA* and 16S *rRNA* for CoPSA and CoNS, respectively, and further for the detection of genes encoding methicillin and vancomycin resistance.

DNA preparation

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, GmbH, Germany) with

modifications from the manufacturer's instructions. Concisely, 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. Then, 200 µl of 100% ethanol was added to the lysate. The rest of the steps were done according to the manufacturer's instructions. Finally, 100 µl of elution buffer provided in the kit were added to the nucleic acid.

PCR amplification

The used primers in this work were obtained from Metabion (Germany) and are listed in Table 1. Primers were used in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Noji-higashi Kusatsu, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was completed in an applied biosystem 2720 thermal cycler. Agarose gel (1.5%) (Applichem GmbH, Darmstadt, Germany) was used to separate the PCR product by electrophoresis in 1× TBE buffer at room temperature using gradients of 5 V/cm. For gel analysis, each gel slot was loaded with 20 µl of the PCR products. To determine the fragment sizes, Gelpilot 100-bp and 100-bp plus DNA ladders (Qiagen, GmbH) were used. Finally, the gel was photographed using a gel documentation system (Alpha Innotech, Biometra, Göttingen, Germany) and the data were analyzed through computer software.

16S rRNA gene sequencing

For purification of PCR products of *16S rRNA* gene, QIA quick PCR Product extraction kit (Qiagen, Valencia, CA) was used. Then, the sequence reaction was performed using Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer), and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (Hitachi, Tokyo, Japan).

Statistical and sequence analysis

Sequence alignments and comparisons were carried out using BIOEDIT (Version 7.0.9.0) [28]. The BLAST algorithm was used to search the NCBI Genbank (http://www.ncbi.nlm.hih.gov/) databases for matching sequences.

Neighbor-joining tree [29] was constructed with the *16s rRNA* gene sequences based on genetic distances, estimated by Kimura's two-parameter method [30], using MEGA 7 [31; http://www.megasoftware.net]. The reliability of the trees was estimated by bootstrap confidence values [32] and 500 bootstrap replications were used.

The neighbor-joining tree was constructed by the following *16s rRNA* gene sequences (by NCBI Genbank accession numbers): CP013943, CP014132, CP022247, KY790453, MF125022, MF319773, MG660861, MG815839, MG911002, LC349807, and LT899997. In addition, the sequences of local isolates are S1-SF, S5-SF, S9-SF, and S15-SF. All of the previous sequences are *S. epidermidis*, except LC349807 and LT899997 are *Staphylococcus* species.

Results

Methicillin- and Vancomycin-resistant Staphylococci in the examined samples

Results presented in Tables 2 and 3 revealed that none of the dairy food samples was contaminated with CoPSA, while 23.3% and 31.7% of samples obtained from food handlers and patients were contaminated with CoPSA, respectively. However, both dairy food, and human samples represented in food handlers and patients were found to be contaminated with CoNS with incidences of 26.7%, 38.3%, and 53.3%, respectively (Tables 2 and 3).

Regarding the screening of the obtained isolates for the antimicrobial resistance, high incidence (81% and 75.4%) of CoPSA isolates obtained from food handlers and patients found to be resistant to methicillin, respectively, as well as 64.3% and 38.6% of the same isolates were determined as vancomycin-resistant, respectively (Table 5). Furthermore, MRCoNS and VRCoNS were detected in dairy foods, food handlers, and patients by 41.7% and 20.8%; 68.0% and 31.9%; and 81.3% and 55.2%, respectively (Table 4 and

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	GCAAAATCCAGCACAACAGGAAACGA CTTGATCTCCAGCCATAATTGGTGG	638	94°C 5 min	94°C 30 sec	55°C 45 sec	72°C 45 sec	72°C 10 min	[25]
16S rRNA	CCTATAAGACTGGGATAACTTCGGG CTTTGAGTTTCAACCTTGCGGTCG	791	94°C 5 min	94°C 30 sec	55°C 45 sec	72°C 45 sec	72°C 10 min	
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310	94°C 5 min	94°C 30 sec	50°C 30 sec	72°C 40 sec	72°C 7 min	[26]
vanA	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	732	94°C 5 min	94°C 30 sec	54°C 45 sec	72°C 45 sec	72°C 10 min	[27]

5). In the present study, resistance to both methicillin and vancomycin antibiotics was recorded in 45.2% and 22.8% of CoPSA strains isolated from food handlers and patients, respectively, and in 18.1%, 21.7%, and 40.6% of CoNS obtained from food, food handlers, and patients, respectively (Table 6).

Table 2. Incidence of CoPSA and CoNS in the examined food samples.

Samples	No. of samples	CoPSA		CoNS	
		No.	%	No.	%
Soft white Cheese	30	0	0	12	40
Processed cheese	30	0	0	7	23
Yoghurt	30	0	0	5	16
Total	90	0	0	24	26.7

Table 3. Incidence of CoPSA and CoNS in the examined human samples.

Samples	No. of samples		CoPSA		CoNS	
			No.	%	No.	%
Food handlers	Hand swabs	30	6	20	10	33.3
	Nasal swabs	30	8	26.7	13	43.3
	Total	60	14	23.3	23	38.3
Patients total	Nasal swabs	30	12	40	18	60
	Diarrhea	30	7	23.3	14	46.7
	Total	60	19	31.7	32	53.3

Table 4. Incidence of MRCoNS and VRCoNS in the examined food samples.

Samples	No. of isolates	MR CoNS		VR CoNS	
		No.	%	No.	%
White cheese	36	16	44.4	13	36.1
Processed cheese	21	12	38.1	2	9.5
Yoghurt	15	6	40	0	0
Total	72	30	41.7	15	20.8

Sequence alignment comparison and Phylogenetic analysis

The sequences obtained in this study showed complete similarities between all local sequences S1-SF, S5-SF, and S15-SF, that obtained from food, hand swabs of food handlers, and diarrhea samples, respectively, and those sequences obtained from the Genbank (CP013943, CP014132, CP022247, KY790453, MF125022, MF319773, MG660861, MG815839, MG911002, LC349807, and LT899997) (Fig. 1). The local isolate S9-SF, which obtained from nasal swabs of patients, showed little variations in the following positions (19, 26, 35, 42, 132, 151, 165, 194, 240, 409, 424, & 430) (Fig. 1); this local sequence needs more investigation with a large number of samples to detect if this variation is true variation or artifact.

The tree was constructed using the nucleotide sequences of 4 *16s rRNA* genes isolated from *S. epidermidis* (local isolates, represented by boxes) and 11 sequences obtained from the Genbank as mentioned before (represented by triangles). All sequences were clipped to an identical length corresponding to the same region before creating the tree (Fig. 2). The tree showed a high degree of similarities between all sequences of local isolates or that imported from the Genbank. The local isolates S1-SF, S5-SF, and S15-SF, that obtained from food, hand swabs of food handlers, and diarrhea samples, respectively, are more close to each other and formed closed lineage in comparison to the other Genbank sequences or the local isolate sequence (S9-SF) that obtained from nasal swabs of patients and formed a separate lineage.

Discussion

Incidence of coagulase positive and negative Staphylococci in different samples

Formerly, CoPSA used to be considerably more critical due to their pathogenic characteristics when compared with CoNS and this has resulted in a limited number of performed studies on the CoNS isolated from foods despite being very common in food, particularly dairy products. Recently, CoNS gained great attention as being

Table 5. Incidence of MRSA, VRSA, MRCoNS, and VRCoNS in the examined human samples.

Samples		CoPSA isolates			CoNS isolates		
		No.	MRSA No. (%)	VRSA No. (%)	No.	MRCoNS No. (%)	VRCoNS No. (%)
Food Handlers	Hand swabs	18	18 (100)	18 (100)	30	20 (66.7)	12 (40)
	Nasal swabs	24	16 (66.7)	9 (37.5)	39	27 (69.2)	10 (25.7)
Total		42	34 (81)	27 (64.3)	69	47 (68)	22 (31.9)
Patients	Nasal swabs	36	28 (77.8)	10 (27.8)	54	43 (79.6)	25 (48.2)
	Diarrheal swabs	21	15 (71.4)	12 (57.1)	42	35 (83.3)	28 (66.7)
Total		57	43 (75.4)	22 (38.6)	96	78 (81.3)	53 (55.2)

the most provident microorganisms in the genus, notably in hospitalized patients causing nosocomial infections in humans [33].

Results obtained in the present study exhibited a higher prevalence of CoNS in soft cheese (31.7%) than earlier reports [34,35]

Regarding contamination of yogurt samples with CoNS; Argaw et al. [36] detected a lower incidence of the organism (2%), and on contrary to this study, Onen and Aygun [37] could not isolate CoNS from yogurt samples.

Contrary to the achieved results, Rodrigues et al. [38] found that 92.0% of the isolates obtained from cheese samples were identified as CoPSA. Also, Carfora et al. [39], Basanisi et al. [40], Ektik et al. [41], Papadopoulos et al. [42], and Usman and Mustapha [43] could isolate CoPSA from dairy products samples, and MRCoPSA and VRCoPSA were among them. The lack of dairy food samples from CoPSA could be contributed to the low pH of the samples as a result of fermentation [44], in addition that food samples originated from large scale factories, while, the occurrence of CoNS in food may result primarily from its ability to withstand adverse environmental conditions throughout the manufacturing, storage and high adaptation capacity of those micro-organisms [45].

Table 6. Incidence of MRS/VRSA and MRCoNS/VRCoNS in the examined samples.

Samples	MR/VR- *S. aureus*	MR/VR-CoNS
Food samples	-	13/72 (18.1%)
Food handlers	19/42 (45.2%)	15/69 (21.7%)
Patients	13/57 (22.8%)	39/96 (40.6%)

When it comes to human health, the role played by food handlers should not be an oversight as they play a great role in the transmission of foodborne diseases, which represent a global health burden, especially transmitting *Staphylococci* either via manual handling or through respiratory secretions during the preparation of food. The presence of CoPSA in food handlers' nasal cavities has been investigated by many authors [46,47]. Abulreesh et al. [46] noted that *S. aureus* could be isolated from nasal cavities in percentages ranged from 20% to 55% in a healthy adult. These data agree with the current work since the microorganism was isolated from 26.7% of the nasal swabs collected from the food handlers, also El Aila et al. [47] reported a comparable result.

A lower incidence of CoPSA was established on the hands (20%) than in the nose of food handlers. This is consistent

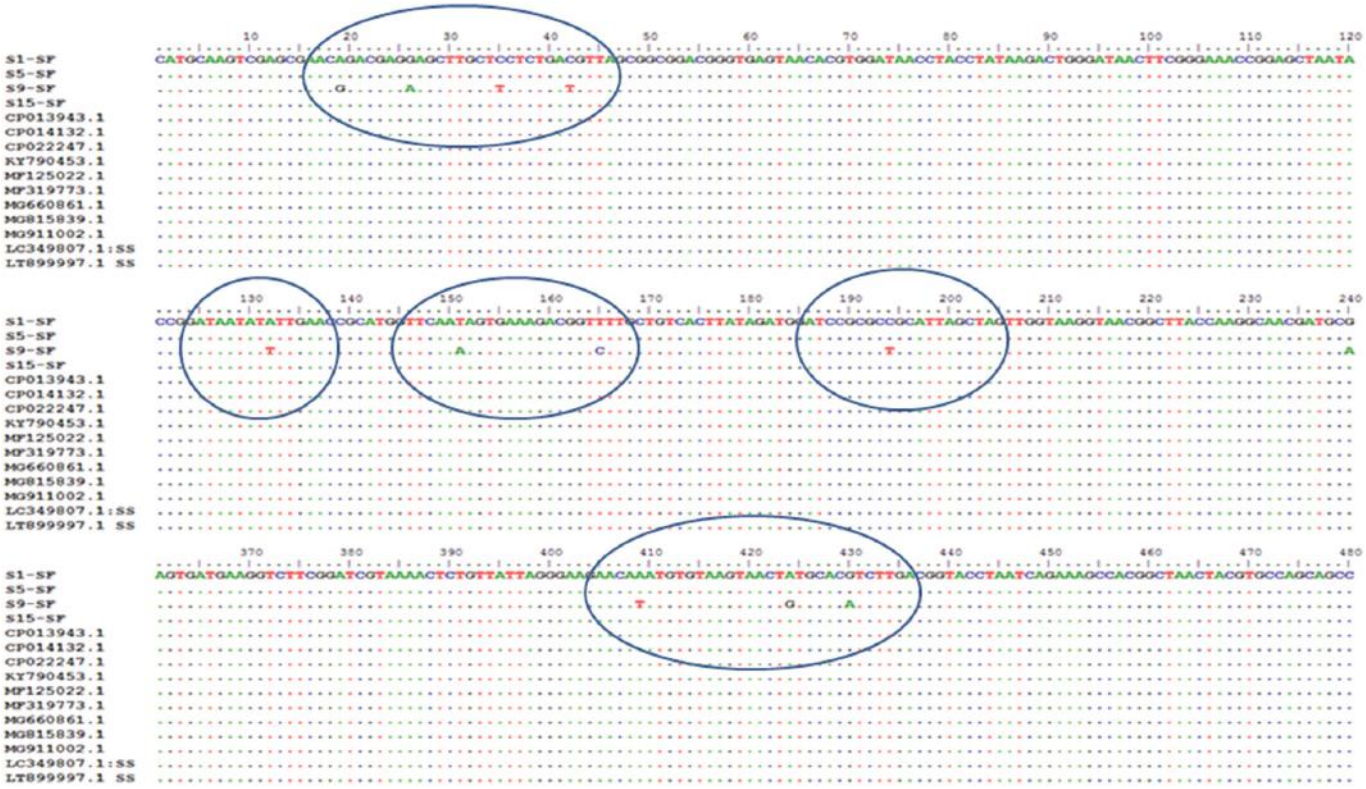

Figure 1. Multiple sequence alignments of *16S rRNA* genes. Dots represent residues identical to the nucleotide in the top sequence. S1-SF: *S. epidermidis* isolated from dairy food; S5-SF: *S. epidermidis* isolated from hand swabs of food handlers; S15-SF: *S. epidermidis* isolated diarrheal swabs of patients and S9-SF: *S. epidermidis* isolated from nasal swabs of patients.

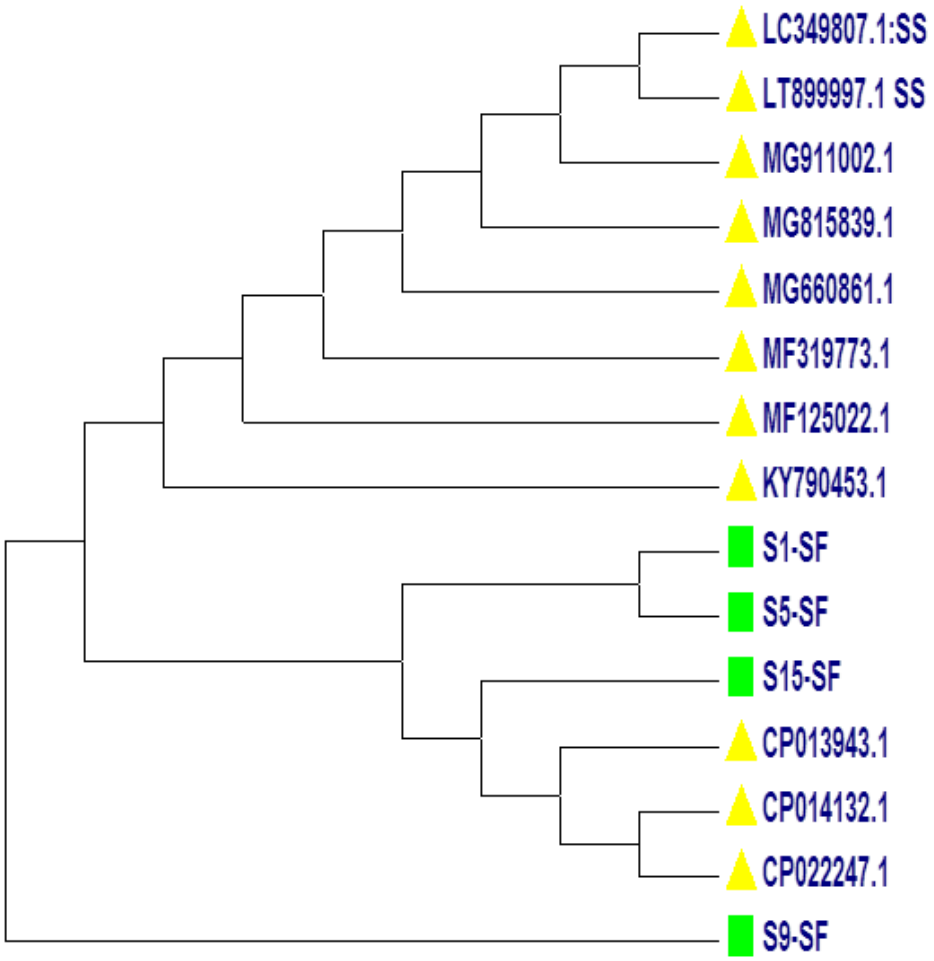

Figure 2. Phylogenetic analysis of the *16S rRNA* gene sequences of CoNS. Phylogenetic tree was constructed by Neighbor-Joining method using the Maximum Composite Likelihood method in MEGA7. S1-SF: *S. epidermidis* isolated from dairy food; S5-SF: *S. epidermidis* isolated from hand swabs of food handlers; S15-SF: *S. epidermidis* isolated diarrheal swabs of patients, and S9-SF: *S. epidermidis* isolated from nasal swabs of patients.

with the findings reported by Çepoğlu et al. [48] and Castro et al. [49]. Contrary results were recorded by Lues and Tonder [50] and Tana et al. [51]. This variation may be due to age, underlying illness, certain behaviors, and the living environment as mentioned by El Aila et al. [47].

Of all the examined 60 food handlers' samples, 38.3% were positive for CoNS, where a higher incidence was found in nasal swabs (43.3%) than hand swabs (33.3%). Closely related result (40%) was isolated from food handlers by Abulreesh and Organji [46] and higher incidence by Çepoğlu et al. [48].

Among hospitalized patients, staphylococcal infection occurs either through food poisoning or from the surroundings (nosocomial infection). According to Herwaldt [52], patients carry CoPSA nasally in a rate ranging from 32% to 82% as a result of possible predisposing factors including immunodeficiency. In this study, CoPSA and CoNS were recovered from 40% and 60% of examined patients' nasal swabs, respectively. Lower incidence was obtained by Kozio-Montewka et al. [53], whereas, it was detected nasally in a higher incidence by Cavalcanti et al. [54]. Furthermore, it was found that 23.3% of the diarrheal samples harbored CoPSA. Closely related result recovered by Cavalcanti et al. [54], while relatively higher incidence obtained by Sung et al. [55]. Also, CoNS was detected in 46.7% of examined samples (Table 3) and a relatively lower result was obtained by Akinkunmi and Lamikanra [56].

Methicillin and Vancomycin resistance in staphylococcal isolates

It is not enough to characterize *Staphylococci* as true MR/VRCoNS or MR/VRCoPSA on the basis of a single test. Both the phenotypic and the genetic tests are demanded in order to accurately screen MRS and/or VRS isolates.

Methicillin resistance determinant *mecA* and vancomycin resistance determinant *vanA* genes were found in all isolates that exhibited phenotypic oxacillin and vancomycin resistance, respectively.

The antibiotic resistance of the group CoNS was not taken into account [16] since for many years, CoNS were estimated as non-pathogenic; in routine laboratory tests, the identification of CoNS usually does not exceed the genus, while coagulase-positive strains are submitted to additional analyses. Resistance to methicillin antibiotic could be detected in 49.1% of 57 CoNS isolates obtained from cheese samples which is greater than the results obtained by Pamuk et al. [34], and lower than the finding reported by Chajęcka-Wierzchowska et al. [45] and Nunes et al. [8].

Since vancomycin has been considered the drug of choice for the treatment of methicillin-resistant *Staphylococci*, so it was important to detect the resistance of the isolates to it. VRCoNS was detected in 26.3% of cheese isolates. Unlike this result, all CoNS strains obtained from cheese samples by Resch et al. [57], Zdolec et al. [58], and Alnakip et al. [59] were sensitive to vancomycin, while Nunes et al. [8] detected a higher incidence of VRCoNS. Moreover, it was found that 40% of CoNS isolated from yogurt samples in this study were resistant to methicillin, while resistance to vancomycin was not exhibited. For the best of our research efforts, there is no available data regarding the drug resistance profile of CoNS isolated from yogurt. These results expanded the current knowledge regarding the microbial quality of hospital food, particularly its contamination with methicillin- and vancomycin-resistant CoNS and its public health importance.

Working in hospitals sounds to be hazardous as a consequence of the carriage of resistant strains by food handlers, as manifested by Ferreira et al. [60] in their study. This study declared that 81% and 64.3% of CoPSA isolates recovered from food handlers showed resistance to methicillin and vancomycin, respectively; from which all isolates recovered from hands were resistant to both antibiotics, while 66.7% and 37.5% of isolates obtained from nasal swabs were resistant to methicillin and vancomycin antibiotics, respectively; Abulreesh and Organji [46] recorded lower incidences of methicillin resistance and higher one of vancomycin resistance (100%) of CoPSA isolates.

On the other hand, CoNS isolated from food handlers showed 68% and 31.9% resistance to methicillin and vancomycin, respectively. Lower resistance to methicillin and higher to vancomycin obtained by Abulreesh and Organji [46]. The widespread of the multidrug-resistant strains among food handlers focus the attention on the sequellae of antibiotic resistance in the health community.

In hospitalized patients, it was noticed that CoPSA recovered from nasal swabs exhibited higher resistance to methicillin (77.8%) than vancomycin (27.8%). Lower incidences of nasal MRSA were detected by Cavalcanti et al. [54]. Concerning CoNS isolates, 79.6% and 48.2% of them showed resistance to methicillin and vancomycin, respectively. Lower incidences of MRCoNS were reported by Cuevas et al. [61] and de la Mària et al. [62], whereas higher VRCoNS incidences were detected by de la Mària et al. [62] and Natoli et al. [63].

Coagulase-positive *S. aureus* (CoSPA) isolated from patients' diarrhea showed resistance to methicillin and vancomycin with incidences of 71.4% and 57.1%, respectively. Mason et al. [25] detected the same percentage of MRCoPSA, whereas all isolates recovered by them were susceptible to vancomycin. Cavalcanti et al. [54] detected a lower incidence of methicillin resistance. Moreover, MR-CoNS and VR-CoNS were recovered from 83.3% and 66.7% of the same diarrheal samples, respectively. Lower findings obtained by Akinkunmi and Lamikanra [56], while El Kholy et al. [64] detected MR-CoNS in 77% of diarrheal samples and all of them were susceptible to vancomycin.

Currently, methicillin-resistant *staphylococci* comprise a very serious obstacle facing the clinicians. MRCoNS group cause cardiovascular, joint, and bloodstream infections, among other conditions [65], while MRSA causes necrotizing pneumonia, severe sepsis, and Waterhouse-Friderichsen syndrome which marked by petechial rash, coagulopathy, and cardiovascular collapse; even apparently healthy hosts can exhibit many of these infections not only the immunosuppressed persons [66]. The methicillin resistance mechanism of CoNS is related to the existence of the *mecA* gene that encodes PBP2a (PBP22'), a penicillin-binding protein, resulting in phenotypic resistance. The *mecA* gene is situated on a mobile genetic element called Staphylococcal Cassette Chromosome *mec* (*SCCmec*). The initial detection of the type IV *SCCmec* cassette in *S. epidermidis* and its later appearance in *S. aureus* over the years introduced CoNS as the source of *SCCmec* and a remarkable factor in the evolution and propagation of a new, potential more resistant MRSA strain through horizontal, interspecies transfer of this element [67–69].

After considering vancomycin as the antibiotic of choice treat MR *staphylococci*, in 1996, the reduced susceptibility to vancomycin in isolates obtained from human has been emerged in Japan raising argument about its efficiency [19]. Since Vancomycin is not regularly used in veterinary medicine [70], so emerging of such vancomycin-resistant staphylococcal strains in the food of animal origin may be as a result of its contamination with vancomycin-resistant isolates originated from human in contact with the food either during its processing or manipulation.

Phylogenetic relationship among methicillin- and vancomycin-resistant CoNS

CoNS strains isolated in the current study were identified as *S. epidermidis*, *S. Saprophyticus*, and *S. xylosus* which obtained from food samples; while, *S. epidermidis*,

S. Schleiferi, *and S. warneri* were isolated from examined human samples. As *S. epidermidis* is the most predominant CoNS species in this study and the most reported CoNS species of clinical significance, sequencing of 4 *S. epidermids* isolates and a phylogenetic tree were constructed. The phylogenetic tree accentuated the close relation between S1-SF, SF5, and S15-SF which obtained from food, hand swabs of food handlers, and diarrhea samples, respectively; while S9-SF occurred in a separated arm was obtained from nasal swabs of patients.

Sequence examination of conserved "housekeeping" genes such as the bacterial *16S rRNA* gene is gradually being used to classify bacterial species in scientific studies. It is useful because the *16S rRNA* gene occurs in all bacteria, is well conserved, and is large enough for informatics resolutions [71]. Multiple sequence alignment and phylogenesis revealed a high degree of similarities between the local isolates and those retrieved from the GeneBank which indicate that *16S rRNA* gene is an excellent tool used for clinical classification and molecular epidemiological studies to distinguish between different bacterial isolates [71,72].

One of the important big obstacles that hinder the overcome of the antimicrobial resistance issue is the insufficiency of information regarding the antimicrobial resistance in food and food animals, but fortunately, there has been marked improvement in the surveillance in these zones in recent years as WHO in collaboration with the Ministry of Health and Population in Egypt conducted a consultative workshop, from 6 to 8 March 2018 in Cairo, to finalize Egypt's national action plan on antimicrobial resistance (AMR) which aligns with that of the WHO to tackle the growing problem of AMR, including resistance to antibiotics, which was endorsed by the World Health Assembly in May 2015. The final draft of the AMR national action plan was discussed with stakeholders such as the Ministry of Agriculture, Ministry of Environment and the Food and Agriculture Organization of the United Nations, as well as experts in human health, animal health, plant production, and the food chain. The plan is based on four pillars: prevention and control of the infection; AMR surveillance; optimizing antimicrobial use, and education and public awareness under the "One Health approach" [73].

Conclusion

The close phylogenetic relation between S1-SF, S5-SF, and S15-SF which obtained from food, hand swabs of food handlers, and patients' diarrheal samples, respectively, highlights the important mutual role can be played by food and food handlers in the epidemiology of *Staphylococci*. This theory can be explained by the presence of *Staphylococci* as commensals of hands and human mucosal membranes, so food handlers can contaminate foods through poor hygienic behavior represented in unsanitary manual contact and respiratory secretions. Also, the presence of coagulase-negative methicillin- and vancomycin-resistant *Staphylococcus* strains in these dairy products presents a risk to public health. Taking the potential implications of the food production chain in the transmission of methicillin- and vancomycin-resistant *Staphylococci* into account, there is a necessity to be constantly monitoring the employers' health and hygiene, application of food safety programs like Hazard Analysis and Critical Control Point (HACCP) system in hospitals during all stages of food manipulation and preparation; and also HACCP system and other quality assurance programs should be designed not only to achieve pathogen reduction goals but also to integrate antimicrobial resistance and prudent use considerations into these programs, and this is considered as a challenge. There is an increasing need for studies on: 1) Microbial resistance in food to improve understanding of the spread of resistance elements and the bacteria that possess them; 2) antimicrobials use in animals to overcome the lack of information in understanding the role of such use in resistance development.

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Conflict of interests

The authors declare that there exists no conflict of interest in the study.

Authors' contribution

The corresponding author designed the plan of the study. Other else, all authors contributed equally to this work and reviewed the final manuscript.

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