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RESEARCH ARTICLE OPEN ACCESS

Prevalence and Genetic Diversity of Coagulase Negative *Staphylococcus* in Food Products Collected from Riyadh Region

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Abstract

The goal of this study was to isolate, count, and identify coagulase-negative *Staphylococci* from meat and dairy products collected in Riyadh, Saudi Arabia, as well as to perform molecular identification of the *mec*A gene. In addition, the prevalence of these bacteria among the examined food products was determined. Ninety samples of both meat and dairy products were collected and examined between February 2018 and August 2019. Mannitol salt agar and VITEK 2 system were carried out and VITEK 2 system was used to identify all bacterial isolates. Also, the molecular technique was used to detect the target gene of *mec*A among CoNS. The proportion of samples in which *Staphylococcus* species isolated is 13.33% (Camel meat), 6.66% (Beef mortadella), 6.66% (Turkish labneh), 33.33% (Cows cheese), 6.66% (Goat labneh), 13.33% (Nabulsy cheese), 13.33% (Haloumi goat cheese) and 6.66% (Akawy white cheese). Counts of coagulase-negative *Staphylococcus* species (as cfu/gm) of sample were around $11x10^4$, $10x10^4$, $9x10^4$, $12x10^4$, $4x10^5$, $11x10^4$ (Nabulsy cheese), $14x10^4$ and $12x10^4$, respectively. The Prevalence of species in both products was 3.30% (*Staphylococcus vitulinus*), 53.30% (*Staphylococcus saprophyticus*), 16.66% (*Staphylococcus hominis*), 6.66%, (*Staphylococcus equorum* and *Staphylococcus lentus*) and 13.33% (*Staphylococcus sciuri*). Furthermore, results showed methicillin specific *mec*A gene was harbored in 40 % of the CoNS.

Keywords: Coagulase-negative *staphylococci*, meats, dairy products

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INTRODUCTION

Staphylococcus spp. could cause food poisoning and a production of enterotoxin in nourishment products.[1] Staphylococcus has different species and divided in two main groups on the basis of coagulase enzyme production; the first group is coagulase-positive *Staphylococci* (CoPS) such as *S. aureus, S. intermedius* and *S. hyicus*.[2] The other group is coagulase negative *Staphylococci* (CoNS) that consist of various species habitat human body.[3] The *Staphylococci* have been frequently isolated from human, animal, and food (diary and meat products).[4]

Staphylococci are foodborne pathogen that are associated with raw unpasteurized milk of cattle suffering from mastitis.[4,5] Meat and dairy products are abundant sources of nutrition such as proteins, fat, minerals, vitamins; and play a significant role in meet nutrient requirements of human.[6] Bacteria causing food poisoning have the ability to habitat animals and might be transmitted to human through the consumption of animal products.[7] Selvaggi study have focused on the resistance of bacteria in domestic animals. Its effect on human health is mostly linked to the misuse of antibiotics.[8]

Recently, CoNS is a typical opportunist and represents one of the main nosocomial pathogens that have a crucial impact on human health[9,10] and associated with high resistance to many antibiotics.[11] The CoNS are important reservoirs of antibiotic resistance genes and associated mobile genetic elements and are believed to contribute to the emergence of successful methicillin resistant *Staphylococcus aureus* (MRSA) clones. Also, multiple antibiotic resistance CoNS were isolated from food and other sources.[9,10] Therefore, the present study aimed to isolate, count and identify CoNS and molecular detection of *mec*A gene from meat and dairy products in Riyadh city, Saudi Arabia.

MATERIAL AND METHODS

Sample collection

To assess the identification of CoNS species and the prevalence rate in meats and dairy products, 45 samples of meat products and 45 samples of dairy products were randomly collected from three local hypermarkets of different three major chains in Riyadh City. All samples were collected in clean, dry and sterile polythene bags and then transported to the laboratory (under cold condition) then refrigerated at 4°C for maximum of 2 hours (hrs) for microbiological analysis and analysis.

Serial dilution preparation of samples

Five gram of meats and dairy products were homogenized with 95 ml sterile distilled water for 2 minutes (Ningbo Sklon, China). Subsequently, 1 ml of the total amount was transferred to 9 ml of sterilized distilled water under sterilized conditions until 10^{-5} dilution.

Enumeration and isolation of *Staphylococci* spp.

Total *Staphylococci* spp. were performed using mannitol salt agar medium. The plates were inoculated with 1 ml^{-1} of homogenate dilutions using surface plating technique. Then, plates were incubated at 37°C for 24-48 hrs. Typical yellow colonies on mannitol salt agar considered as mannitol fermenter *Staphylococci* spp.

Purification of isolated strains

A typical colony of selected Staphylococcus spp. heavily streaked on mannitol salt agar and then incubated at 37°C for 24 hrs. Bacterial isolates were enriched in nutrient broth containing 30% (v/v) glycerol (BDH Chemicals Ltd. Pool, England) and then stored at −80°C for further analysis.

Inoculum preparation

Each organism suspension was prepared from growth of pure cultures of bacteria cultivated on plates containing nutrient agar. Then they were incubated overnight at 37°C. Bacterial cells were suspended in 2.5 ml of a 0.45% sodium chloride solution. The suspension used in the VITEK 2 system (bioMerieux) was adjusted to a McFarland standard of 0.5 by using a Denscheck. Suspensions used for the comparative identification method were made according to the manufacturer's instructions.

Biochemical tests of isolated Staphylococcus spp. with the VITEK 2 System

The VITEK 2 system was used according to the manufacturer's instructions; ID-Gram Positive Cocci cards (ID-GPC cards; bioMérieux) were used for identification. The ID-GPC card is a 64-well plastic card containing 18 empty wells and 46 wells for fluorescent and inhibitory tests. Final results were obtained automatically. All used cards were automatically discarded into a waste container.

Table 1. Bacterial isolates in food products that produce catalase enzymes

	Samples	Bacterial isolates	Catalase test	Coagulase test
Dairy Products	Turkish labneh	Isolate 1	+ve	-ve
	Cow cheese	Isolate 2	+ve	-ve
	Goat labneh	Isolate 3	+ve	-ve
	Nabulsy cheese	Isolate 4	+ve	-ve
	Haloumi goat cheese	Isolate 5	+ve	-ve
	Akawy white cheese	Isolate 6	+ve	-ve
Meat Products	Camel meat	Isolate 7	+ve	-ve
		Isolate 8	+ve	-ve
		Isolate 9	+ve	-ve
	Beef mortadella	Isolate 10	+ve	-ve

+ve: positive test, -ve: negative test

Table 2. Identification of bacterial isolates from dairy and meat products with VETIK2 system

	Samples	Number of isolates	Species	Identification percentage
Dairy Products	Turkish labneh	Isolate 1	*S. saprophyticus*	96.5%
	Cow cheese	Isolate 2	*S. saprophyticus*	96.5%
	Goat labneh	Isolate 3	*S. lentus*	97 %
	Nabulsy cheese	Isolate 4	*S. hominis*	96.9%
	Haloumi goat cheese	Isolate 5	*S. sciuri*	98 %
	Akawy white cheese	Isolate 6	*S. saprophyticus*	96.5%
Meat Products	Camel meat	Isolate 7	*S. vitulinus*	99 %
		Isolate 8	*S. saprophyticus*	96.5%
		Isolate 9	*S. hominis*	96.9%
	Beef mortadella	Isolate 10	*S. equorum*	99 %

Molecular detection of *mec*A gene in the recovered coagulase negative *Staphylococci*

To determine the targeted gene in CoNS, as a reservoir for antibiotics resistance genes, two primer sets previously reported Moussa and Shibl 200912 The primers were used for the detection of the *mec*A gene, that amplifies 1319 bp fragments specific for *mec*A gene.

RESULTS

The prevalence of CoNS

Ninety samples were collected and examined microbiologically to isolate *Staphylococcus* species. Out of 90 samples, *Staphylococci* were isolated from 30 samples (33.33%). Of 30 samples, 6 collected from meat products (6.66%) and 24 from dairy products (26.66%). All bacterial isolates were tested for catalase and coagulase activity to confirm isolates belong to *Staphylococci* group., All bacterial isolates were positive for catalase and negative for coagulase (Table 1). The proportion of CoNS isolates in type of samples and source varied. The predominant species was *S. saprophyticus* (n=16; 53.33%) followed by *S. hominis* (n= 5; 16.66%), *S. sciuri (n=4; 13.33%), both of S. equorum* and *S. lentus (n= 2 each; 6.66%), S. vitulinus* (n= 1 each; 3.33%) (Table 4). By VITEK 2 equipment, all isolates were identified as species of *Staphylococci*, named as 99% of *Staphylococcus vitulinus*, 96.5% of *Staphylococcus saprophyticus*, 96.9% of *Staphylococcus hominis*, 99% of *Staphylococcus equorum*, 97% of *Staphylococcus lentus*, 98 % of *Staphylococcus sciuri* (Table 2).

CoNS isolates from meat products

Of 45 samples, the proportion of 6

isolates that identified as Staphylococcus species using VITEK 2 were 13.33%. The positive samples of camel meat (n=4) and beef mortadella (n=2) were 8.89% and 4.44%, respectively. Both *S. saprophyticus* (n=2) and *S. equorum* (n=2) were predominant (4.44% each) in this product followed by *S. hominis* and *S. Vitulinus* (2.22% each). Of 6 isolates, both *S. saprophyticus* and *S. equorum* were predominant (33.33% each) in this product followed by *S. hominis* and *S. Vitulinus* (16.66% each) (Table 4). *Staphylococcus* spp. counts in meat products were $9x10^4$ cfu/gm (*S. vitulinus*), $12x10^4$ cfu/gm (*S. saprophyticus*), $13x10^4$ cfu/gm (*S. hominis*) in camel meat. and $10x10^4$ cfu/gm (*S. equorum*) in beef mortedella (Table 3).

CoNS isolates from dairy products

As stated above in dairy products samples, 53.33% of which (24 out of 45) was positive for the isolation of CoNS isolates. The proportion of different dairy products was 4.44% (Turkish labneh; n=2), 22.22% (Cow cheese; n=10), 4.44% (Goat labneh; n=2), 8.89% (Nabulsy cheese; n=4), 8.89% (haloumi goat cheese; n=4), 4.44% (Akawy white cheese; n=2). The higher proportion of dairy sample in which *Staphylococcus* species were isolated was noticed in Cow cheese (33.33%),

Table 3. Total counts of *Staphylococcus* spp. in meat and dairy products (CFU/gm)

	Samples	Bacterial isolates	CFU/gm
Dairy Products	Turkish labneh	*S. saprophyticus*	$9*10^4$
	Cow cheese	*S. saprophyticus*	$12*10^4$
	Goat labneh	*S. lentus*	$4*10^5$
	Nabulsy cheese	*S. hominis*	$11*10^4$
	Haloumi goat cheese	*S. sciuri*	$14*10^4$
	Akawy white cheese	*S. saprophyticus*	$12*10^4$
Meat Products	Camel meat	*S. vitulinus*	$13*10^4$
		S. saprophyticus	$12*10^4$
		S.hominis	$9*10^4$
	Beef mortadella	*S. equorum*	$10*10^4$

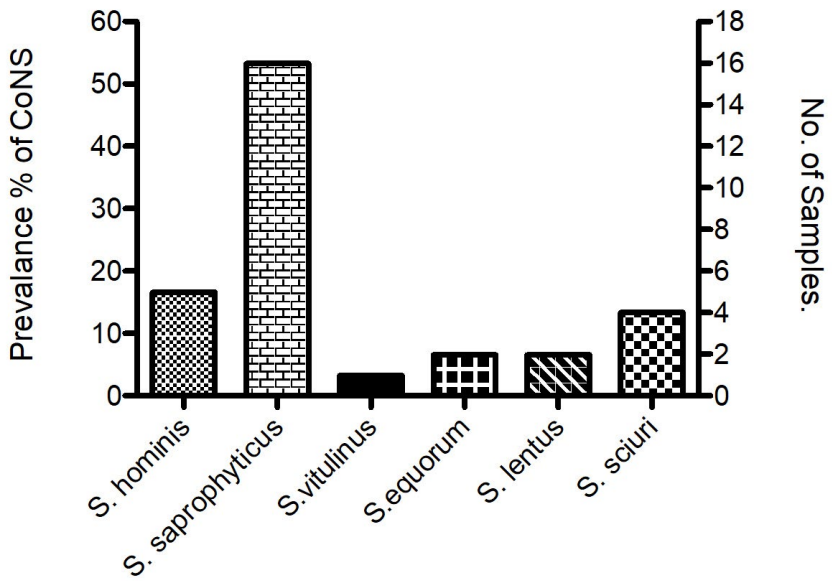

Fig. 1. Prevalence rate of different CoNS isolated from targeted products.

but Turkish labneh, Goat labneh and Akawy white cheese have lowest proportion of isolates (8.33% each). Of 24 isolates, the higher prevalence of CoNS isolates among diary samples was *S. saprophyticus* (n=14; 58.33%), followed by *S. hominis (n=4; 16.66%)* and *S. sciuri* (n=4; 16.66%), then *S. lentus* (n=2; 8.88%) (Table 4, Fig. 1). *Staphylococcus* spp. counts were $9x10^4$ cfu/gm (*S. saprophyticus*) in Turkish labneh, $12x10^4$ cfu/gm (*S. saprophyticus*) in Cow Cheese, $4x10^5$ cfu/gm (*S. lentus*) in Goat labneh, $11x10^4$ cfu/gm (*S. hominis*) in Nabulsy cheese, $14x10^4$ cfu/gm (*S. sciuri*) in haloumi goat cheese and $12x10^4$ cfu/gm (*S. saprophyticus*) in Akawy white cheese (Table 3).

The detection of *mec*A gene in the recovered CoNS

Agarose gel electrophoresis shows amplification of 1319 bp fragments of *mec*A gene of CoVS. Fourthly percent (40%) of the CoNS were positive and harboring the coagulase gene and amplification of 1319 bp fragments were observed (Fig. 2).

DISCUSSION

CoPS and CoNS are both considered as commensal opportunistic infections of human and animal.[13] These species are known to cause wound infection in human and mastitis and skin infection in animals.[14] Also, they can cause food poisoning through secretion of enterotoxins.[15] Sometime the acquisition of antimicrobial resistance might be occurred.[16] This study was evaluating the prevalence of *Staphylococcus* spp. in retail markets of meat and dairies. Other species of *Staphylococcus* were found, but *S. aureus* was not. Different CoNS were isolated and identified by

Table 4. Distribution of CoNS isolates in food products and in which sample isolated

	Samples	Bacterial isolates	No. (%) of isolates in 45 samples	% of isolates in 30 samples
Dairy Products	Turkish labneh	*S. saprophyticus*	2 (4.4)	6.6
	Cow cheese	*S. saprophyticus*	10 (22.2)	33.3
	Goat labneh	*S. lentus*	2 (4.4)	6.6
	Nabulsy cheese	*S. hominis*	4 (8.8)	13.3
	Haloumi goat cheese	*S. sciuri*	4 (8.8)	13.3
	Akawy white cheese	*S. saprophyticus*	2 (4.4)	6.6
Meat Products	Camel meat	*S. vitulinus*	1 (2.2)	3.3
		S. saprophyticus	2 (4.4)	6.6
		S.hominis	1 (2.2)	6.6
	Beef mortadella	*S. equorum*	2 (4.4)	3.3

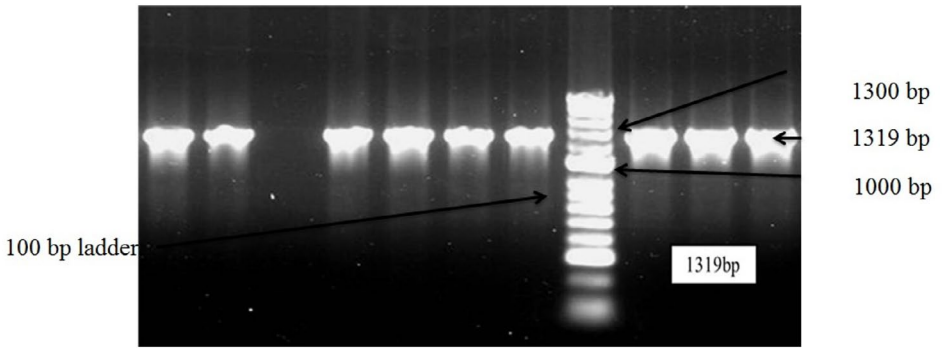

Fig. 2. Agarose gel electrolysis. The gel shows amplification of 1319 bp pair fragments of *mec*A gene. Lanes 1,2 (from left) shows coagulase negative, while lane 3 showing negative samples, and lane 8 shows 100bp ladder. Lanes 4,5,6,7,9,10,11 shows positive amplification of 1319 bp of Staphylococcus species harboring *mec*A gene.

VITEK 2 system as *S. vitulinus, S. saprophyticus, S. hominis, S. quorum, S. lentus, S. sciuri* from dairy and meat products.[17-19]

In this study, a total of 30 samples (33.3%) CoNS were isolated from 90 tested samples of meat and dairy products. The proportion of CoNS in both products was 6.6% and 26.6%, respectively. The higher prevalence of CoNS isolates in diary samples was *S. saprophyticus* (58.33%) followed by *S. hominis* and *S. sciuri* 16.66% each, then the lower isolates of CoNS was *S. lentus* (8.88%). We have found that that CoNS was significantly exist in meat and dairy products without existence of CoPS in the same products. That might provide a significant association between *staphylococci* and live stock.[13] As reported in different study, the standard hygienic process in dairy farms usually conducted,[20] but the high prevalence might be attributed to insufficient standard hygiene during the process or following the distribution of food products in retail markets. Moreover, the poor quality of milking and udder preparation might play an important role in contamination during the milking process.[13,21]

Our findings demonstrated that the prevalence rate of *Staphylococcus* spp. in meat product was 13.3% and the camel meat has the highest contamination rate of *Staphylococcus* spp. (8.9%) of all analyzed samples, including *S. vitulinus, S. saprophyticus* and *S. hominis* (Table 2). Similarly, the poor hygienic process during or post slaughtering.[13,22] and transportation of meat product to different retails markets might be considered. In comparison to meat products, the dairy products have higher prevalence rate of of *Staphylococcus* spp. (53.33%). Our study supports the findings of other authors who isolated CoNS from milk. However, their report disclosed toxigenic strains of *Staphylococci* in goat milk.[23] As reviewed, the isolates from 15-55% from dairy products produce enterotoxin.[24]

We have found that that CoNS was significantly exist without existence of coagulase positive *Staphylococci* in meat and dairy products. Another study claimed that the dairy products that contains CoNS is tend to be safe in regards to health hazard associated with CoNS.[25] Vasileiou et. al., have isolated and studied epidemiological characterizations of *S. saprophyticus S. xylosus, S. carnosus* and *S. equorum* from a wide range of foods products such as meat, cheese and milk.[9] The existence numbers of these bacteria might attribute to inappropriate heat treatment and temperatures; and poor hygiene during food processing or after the food production due to contamination after pasteurization.[26] Furthermore, *S. saprophyticus* was the highest (n= 16; 53.3%) in food products (Fig. 1). S. saprophyticus was the most predominant species of CoNS in dairy products with approximate count ($11*10^4$ cfu/gm). However, the highest growth of species was *S. lentus* with average count $4*10^5$ cfu/gm (Table 3). However, *S. vitulinus* was the lowest prevalence (n=1; 3.3%) among these products (Fig. 1). The existence of of *Staphylococci* species in food product seems to have their ability to colonize and cause a disease in different hosts.[27]

Our results demonstrated that 40% of the CoNS harboring the gene of *mec*A specific for methicillin resistance (Fig. 2). It suggested that the cause of methicillin resistance might be attributed to the mechanisms rather than expression of *mec*A.[28] There are some findings of other authors who detected antibiotics resistance of CoNS which were isolated from various meat and dairy products.[17-19] As stated in other studies that CoNS derived from camel milk was significantly resistance to around 70% of antibiotics such as erythromycin, colistin sulphate, penicillin G, oxacillin and vancomycin. On the other hand, CoPS of paired samples have showed resistance to only 3 antibiotics used such as penicillin G, oxacillin and vancomycin. Taken together, all isolates of CoNS from dairy products showed a full resistance to main antibiotics of penicillin G and oxacillin.[3] Also, the CoNS might be easily developing to antibiotics resistant than *S. aureus*.[29]

Collectively, this study is considered as a key work to limit the prevalence of the CoNS and reduce the microbiological risk to the minimum. This will be approached through monitoring the animals and improving food safety as well.[30] The existence of Satphylococci species in meat & dairy products might have the probability to serve as a transmission factors to spread more pathogenic and severe strains in our community.[7] The contamination of retail meat and dairy products with Satphylococci species might be due to food production line and insufficient hygiene of food process that likely happened before marketing.

CONCLUSIONS

All samples isolated from meat and dairy products showed a significant existence of CoSN with around 33.3%. Moreover, not all the isolated CoNS from different food samples were resistant to methicillin as only 40% of which harboring *mec*A gene. As a conclusion, survey and diagnostic investigations are suggested as alternative solutions. The epidemiology of coagulase negative *Staphylococci* isolates from dairy and meat products is significant, and it should be incorporated in public health, food safety, and hygiene efforts.

Limitations

The number of collected samples is considered very low (90 samples of meat and diary products) to identify the the prevalence rate, as the negative samples of food product were high. Thus, more samples are required to be collected in the future and should be collected from different sources and different regions to make more comprehensive study. Furthermore, different antibiotics should be utilized to identify the resistance of antibiotics.

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DATA AVAILABILITY

The datasets generated during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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