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# The Prevalence of Multidrug-resistant Staphylococci in Food and the Environment of Makkah, Saudi Arabia

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## ABSTRACT

The aim of this survey was to investigate the incidence of *Staphylococcus aureus* and coagulase-negative staphylococci and their antibiogram in food and the environment in Makkah, western Saudi Arabia. Staphylococci were detected using selective plating, employing *Staphylococcus* medium no. 110 and mannitol salt agar. Confirmation of isolates was carried out using Gram staining, production of catalase, coagulase and type of blood heamolysis. Disk diffusion method was used to test susceptibility patterns of staphylococci against seven antibiotics. The results showed that staphylococci were present in almost all types of samples. *Staph. aureus* were prevalent in raw milk, cheese, mucous membranes and to a degree, in biofilm. Multidrug-resistance was noted in both coagulase-positive and coagulase-negative isolates from all types of samples. A remarkable level of resistance to beta-lactams and glycopeptides was exhibited by *Staph. aureus* and coagulase-negative staphylococci recovered from all types of samples. The results reported in this study clearly showed the wide spread of multidrug-resistant *Staph. aureus* and coagulase-negative staphylococci in food and the environment and highlighted their public health hazards. To our best knowledge this study is the first to provide evidence of the existence of multidrug-resistant *Staph. aureus* and coagulase-negative staphylococci in food and the environment in Makkah, western Saudi Arabia.

**Key words:** Antibiotic resistance, biofilm, foodstuffs, environment, public health, *Staphylococcus*

## INTRODUCTION

The staphylococci are Gram-positive spherical cells, usually arranged in grapelike irregular clusters. The genus *Staphylococcus* has at least 40 species which are separated in to two major groups on the basis of their ability to clot (coagulate) blood plasma by the action of staphylocoagulas (Somerville and Proctor, 2009). The coagulase-positive staphylococci (CoPS) include pathogenic species such as *Staph. aureus*, while the coagulase-negative staphylococci (CoNS) include species that are part of the normal flora of the skin in humans such as *Staph. epidermidis* (Casey *et al*., 2007). Staphylococci are ubiquitous in the environment and found as part of the normal flora in soil, water, skin and mucous membranes of humans and warm-blooded animals and have been frequently isolated from a wide range of foodstuffs such as dairy products and meat (Irlinger, 2008).

The coagulase-positive *Staphylococcus aureus* is a major cause of various community and hospital acquired infections (Goering, 2008). It causes skin and soft tissues infections, surgical site infections and bone and joint infections (Casey *et al*., 2007; Ippolito *et al*., 2010;

Kluytmans, 2010). *Staphylococcus aureus* is a common cause of hospital-acquired bacteraemia and it is associated with hospital-acquired respiratory tract infections (Casey *et al*., 2007; Ippolito *et al*., 2010). It is an important food-borne pathogen that usually associated with raw unpasteurized milk of dairy cattle suffering staphylococcal-associated mastitis (Irlinger, 2008; Morgan, 2008). The nasal carriage of *Staph. aureus* in healthy adults was reported to be around 30% in the population (Kluytmans, 2010).

The coagulase-negative staphylococci, are common components of the human skin microflora and play an important role in flavor and aroma formation through the production of fermented foods, such as cheese and sausage. In recent years, there has been an increase in cases of nosocomial infections in which coagulase-negative staphylococci are implicated (Casey *et al*., 2007; Irlinger, 2008, Piette and Verschraegen, 2009).

Antibiotic resistant staphylococci are major public health concern since the bacteria can be easily circulated in the environment. Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) have increased world-wide during the past twenty years (Deresinski, 2005; Ippolito *et al*., 2010). Multiple drug-resistant *Staph. aureus* have been frequently recovered from foodstuffs (Normanno *et al*., 2007a), biofilm formation (Lancellotti *et al*., 2007), nasal mucosa of humans (Acco *et al*., 2003), clinical cases (Stefani and Goglio, 2010) and livestock (Wulf and Voss, 2008). As regards coagulase-negative staphylococci, methicillin-resistant *Staph. epidermidis* (MRSE) have been increasingly found to be associated with noscomial infections (Casey *et al*., 2007). Multiple antibiotic resistant CoNS were also recovered from food and potable water (Faria *et al*., 2009; Sawant *et al*., 2009).

In Saudi Arabia, the incidence of community and hospital-acquired MRSA is well documented (Bukharie and Abdelhadi, 2001; Asghar and Momenah, 2005; Baddour *et al*., 2007; Bukhari and Al-Otaibi, 2009; Bukharie, 2010). Few reports focused on bacterial food poisoning, showed that staphylococci are implicated in 41% (n = 6052) of bacterial food poisoning cases in Saudi Arabia (Al-Mazrou, 2004). Despite all these reports, very little is known about the prevalence of drug resistant staphylococci in foodstuffs and the environment in Saudi Arabia, particularly in the city of Makkah.

Therefore, the aim of this study was to investigate the prevalence of coagulase-positive and coagulase-negative staphylococci and their antibiogram in dairy, drinking water, potable water-associated biofilm and food handlers in Makkah, western Saudi Arabia.

## MATERIALS AND METHODS

**Sampling:** A total of fifty nine different samples were screened for the presence of coagualse-positive (CoPS) and coagulase-negative (CoNS) staphylococci and their antibiogram, during the month of December 2010. Samples were nine ready-to-drink raw milks. Cheese samples were twelve in total. Ten samples of potable water. Biofilm samples were nine taps swab and nine tap-filters swab, eighteen samples in total and ten samples of nasal swabs of food handlers. Microbiological analysis of all samples was begun on the same day of sampling.

Three samples of each of raw bovine, caprine and camel milk were examined. Each sample was collected from different locations, i.e., nine different locations (3 samples from 3 locations). These samples were purchased from farms. Usually ready-to-drink raw milk is sold by farms in disposable screw-top plastic bottles.

Cheese samples were three Hungarian-imported feta cheese (white cheese), three Egyptian-imported romy cheese (yellow cheese), three white processed cheese and three yellow processed

cheese. All samples were purchased from local supermarkets, feta and romy cheese were transferred to the laboratory in sterile bags.

Potable water samples, ten in total, were collected from drinking water tankers, which are used to provide potable water for private households. Samples were collected using sterile 200 mL screw-top bottles.

Biofilm samples were simply nine samples of taps swab and nine samples of tap-filters swab. In brief, three different taps were internally swabbed using sterile cotton swabs from three different households (3×3). Another three different taps with filters were swabbed from the same households (3×3), the filters were removed from the taps and the clear formation of biofilm on the interior part of the filters was swabbed.

The anterior nares of ten food handlers of a local food industry, located in the outskirts of Makkah city were sampled using sterile cotton swabs.

**Isolation of staphylococci from raw milk and cheese:** Each milk sample was serially diluted using sterile deionized water and a volume of 1.0 mL was plated onto *Staphylococcus* medium No. 110 agar plates (Oxoid, Basingstoke, UK). All determinations were made in duplicates. Incubation of plates was achieved under aerobic conditions at 35°C for 48 h. For each cheese sample, 10 g was weighted and dispersed aseptically in 90 mL of sterile deionized water and homogenized in a sterile polyethylene bag using a stomacher 400 circulator (Seward, Worthing, UK) for 2 min. Serial dilutions were made in sterile deionized water and the procedure was then continued as described with milk samples.

**Isolation of staphylococci from potable water:** Membrane filter technique was employed for the detection of staphylococci in potable water. Briefly, 100 mL of water was filtered through 0.45 μm, diameter 47 mm, cellulose nitrate and mixed ester membrane filters (Camlab, Cambridge, UK). The membranes were then placed on *Staphylococcus* medium No. 110 agar plates (Oxoid). Incubation was achieved aerobically as described above.

**Isolation of staphylococci from potable water-associated biofilm:** All taps swab and tap-filters swab were streaked onto *Staphylococcus* medium No. 110 agar (Oxoid), incubation of plates was performed as described above.

**Isolation of staphylococci from the nares of food handlers:** All nasal swabs were streaked onto mannitol-salt agar (Oxoid). Plates were incubated under aerobic conditions for 48 h at 35°C.

**Identification of isolates:** From each plate, three typical colonies of staphylococci were isolated and cultured separately on sheep blood agar (Saudi Prepared Media Laboratory, Riyadh, Saudi Arabia). The identification was carried out using the following tests: Gram staining, production of catalase, coagulase using the BBL Staphyloslide latex test (Becton, Dickinson and Company, Maryland, USA)and observation of type of haemolysis on blood agar. The *Staph. aureus* NCTC 12989 was used as a control strain.

**Antibiotic susceptibility tests:** Antibiotic susceptibilities were tested by applying the disk diffusion method according to the guidelines of the British Society for Antimicrobial Chemotherapy (BSAC, 2010) using Mueller-Hinton agar (Oxoid) (Siddiqi *et al*., 2002). Seven commercial

sensitivity disks (Mast Diagnostics, Bootle, UK) were used: Erythromycin (60 μg $mL^{-1}$), Rifampicin (15 μg $mL^{-1}$), Colistin sulphate (10 μg $mL^{-1}$), Penicillin G (2 IU), Kanamycin (1000 μg $mL^{-1}$), Vancomycin (5 μg $mL^{-1}$) and Oxacillin (1 μg $mL^{-1}$) (Bio-Rad, Hercules, USA). *Staphylococcus aureus* NCTC 12989 served as a control to ensure the accuracy of testing.

**Statistical analysis:** To test the hypothesis that the abundance of staphylococci should be similar in all three different types of raw milk, Kruskal-Wallis non-parametric test was used. This test allows the comparison between more than two samples. On the other hand, to compare the abundance of staphylococci in two different types of cheese, the non-parametric Mann-Whitney U-test (two-tailed) was performed. Non-parametric tests are generally used with data that are not normally distributed.

## RESULTS

**The prevalence of staphylococci in raw milk and cheese:** staphylococci were recovered from all bovine and camel raw milk samples (100%), but not all caprine raw milk (66.6%). Higher prevalence of staphylococci was noted in bovine raw milk were the mean colony count was about $5.7\times10^3$ cfu $mL^{-1}$, while caprine milk had mean colony count of $0.009\times10^3$ cfu $mL^{-1}$. With regard to cheese, processed cheese (both white and yellow) yielded no staphylococci. However, Egyptian-imported romy cheese had mean counts of $2.83\times10^3$ cfu $g^{-1}$ of staphylococci (Table 1). Kruskal-Wallis non-parametric test revealed a statistical significant difference ($p = 0.001$) in the prevalence of staphylococci between the three different types of raw milk, where staphylococci were more abundant in bovine raw milk (Table 2). In the same way, using the Mann-Whitney U test (two-tailed), it was found that Staphylococci were significantly abundant in romy cheese ($p = 0.0051$) compared with feta cheese (Table 3).

Coagulase-positive staphylococci (CoPS) were detected in all samples of bovine and camel raw milk and romy cheese, however, only one sample of caprine raw milk yielded CoPS and none of the feta cheese samples yielded CoPS (Table 4).

**The prevalence of staphylococci in potable water, biofilm and the nares of food handlers:** Of the total ten samples of potable water, Staphylococci were recovered from two

Table 1: The prevalence of staphylococci in food and environment in Makkah, Saudi Arabia

| | | Colony count of staphylococci |
|---|---|---|
| Type of sample | N/P (%) | Mean (range) n* |
| Raw bovine milk ($\times10^3$ cfu $mL^{-1}$) | 3/3 (100) | 5.7 (1.0-9.0) 6 |
| Raw camel milk ($\times10^3$ cfu $mL^{-1}$) | 3/3 (100) | 1.06 (0.1-2.7) 6 |
| Raw caprine milk ($\times10^3$ cfu $mL^{-1}$) | 3/2 (66.7) | 0.009 (0.0-0.017) 6 |
| White processed cheese ($\times10^3$ cfu $g^{-1}$) | 3/0 (0) | 0.0 (0.0-0.0) 6 |
| Yellow processed cheese ($\times10^3$ cfu $g^{-1}$) | 3/0 (0) | 0.0 (0.0-0.0) 6 |
| Romy cheese (yellow) ($\times10^3$ cfu $g^{-1}$) | 3/3 (100) | 2.83 (1.0-5.2) 6 |
| Feta cheese (white) ($\times10^3$ cfu $g^{-1}$) | 3/3 (100) | 0.010 (0.002-0.013) 6 |
| Potable water (cfu 100 $mL^{-1}$) | 10/2 (20) | 8.0 (0.0-11) 10 |
| Taps swab | 9/5 (55.6) | N/A |
| Tap-filters swab | 9/6 (66.7) | N/A |
| Nasal swabs | 10/10 (100) | N/A |

N: Total No. of samples, P: Total No. of positive samples, n* : No. of replicates plates, N/A: Not available

Table 2: The prevalence of staphylococci in different types of raw milk in Makkah, Saudi Arabia

| | Mean (range) n | | | |
|---|---|---|---|---|
| | Raw bovine milk | Raw camel milk | Raw caprine milk | p-value |
| Staphylococci ($\times 10^3$ cfu $mL^{-1}$) | 5.7 (1.0-9.0) 6 | 1.06 (0.1-2.7) 6 | 0.009 (0.0-0.017) 6 | 0.001 |

N: No. of plates replicate, p is the probability that there is no difference in colony counts of staphylococci between different types of raw milk (Kruskal-Wallis test)

Table 3: The prevalence of staphylococci in different types of imported cheese in Makkah, Saudi Arabia

| | Mean (range) n | | |
|---|---|---|---|
| | Romy cheese | Feta cheese | p-value |
| Staphylococci ($\times 10^3$ cfu $g^{-1}$) | 2.83 (1.0-5.2) 6 | 0.010 (0.002-0.013) 6 | 0.005 |

N: No. of plates replicate, *P* is the probability that there is no difference in colony counts of staphylococci between romy and feta cheese (Mann-Whitney U test, two-tailed)

Table 4: The prevalence of coagulae-positive staphylococci in food and the environment in Makkah, Saudi Arabia

| | N/P (%) | No of sample positive for CoPS (%) |
|---|---|---|
| Raw bovine milk | 3/3 | 3.0 (100) |
| Raw camel milk | 3/3 | 3.0 (100) |
| Raw caprine milk | 3/2 | 1.0 (50) |
| Romy cheese | 3/3 | 3.0 (100) |
| Feta cheese | 3/3 | 0.0 (0.0) |
| Potable water | 10/2 (20) | 0.0 (0.0) |
| Taps swab | 9/5 (55.6) | 1.0 (2.0) |
| Tap-filters swab | 9/6 (66.7) | 2.0 (33.3) |
| Nasal swabs | 10/10 (100) | 6.0 (60) |

N: Total No. of samples, p: Total No. of positive samples for staphylococci, (%) percentage of positive samples

samples only (20%). The prevalence of staphylococci was shown to be low since the mean colony count was around 8.0 cfu 100 $mL^{-1}$. With regard to the incidence of staphylococci within biofilm formation, 55.5 and 66.6% of taps swab and tap-filters swab respectively yielded staphylococci (Table 1). No CoPS were detected in potable water. However, the incidence of CoPS in taps swab and tap-filters swab was 2.0 and 33.3%, respectively. All nasal swabs of ten different food handlers yielded staphylococci (100%) and the incidence of CoPS in the nasal mucosa of food handlers was observed in 60% of the samples (Table 4).

**Antibiotic susceptibilities of CoPS and CoNS isolated from food and the environment of Makkah, Saudi Arabia:** Antibiotic susceptibility patterns of CoPS and CoNS isolates from raw milk, cheese, water and biofilm are listed in Table 5. All CoPS isolates from different types of raw milk, romy cheese and biofilm were resistant to both penicillin G and oxacillin, while resistance to vancomycin was only observed in isolates derived from camel raw milk, romy cheese and biofilm samples. Biofilm derived CoPS isolates were resistant to four out of the seven antibiotics used. With regards to CoNS isolates from food, water and biofilm showed resistance to penicillin G and oxacillin. Resistance to vancomycin was noted in CoNS recovered from bovine and camel raw milk, feta cheese, drinking water and biofilm. Remarkably, CoNS isolates from camel raw milk and potable water were sensitive to only two out of the seven antibiotics used (Table 5).

Table 5: Antibiotic susceptibilities of staphylococci isolated from food and environmental samples in Makkah, Saudi Arabia

| CoPS** | E | RP | CO | PG | OX | K | VA |
|---|---|---|---|---|---|---|---|
| Raw bovine milk | S | S | S | R | R | S | S |
| Raw camel milk | S | S | S | R | R | S | R |
| Raw caprine milk | S | S | S | R | R | S | S |
| Romy cheese | S | S | S | R | R | S | R |
| Taps swab | S | S | R | R | R | S | R |
| Tap-filters swab | S | S | R | R | R | S | R |
| NCTC 12989† | S | S | S | R | S | S | S |
| CoNS** | E | RP | CO | PG | OX | K | VA |
| Raw bovine milk | S | S | R | R | R | S | R |
| Raw caprine milk | R | S | S | R | R | S | S |
| Raw camel milk | R | S | R | R | R | S | R |
| Romy cheese | S | S | S | R | R | S | S |
| Feta cheese | S | S | R | R | R | S | R |
| Potable water | IR | S | R | R | R | S | R |
| Taps swab | S | S | R | R | R | S | R |
| Tap-filters swab | S | S | R | R | R | S | S |

S: Sensitive, R: Resistant, IR: Intermediate resistance, E: Erythromycin, RP: Rifampicin, CO: Colistin sulphate, PG: Penecillin G, OX: Oxacillin, KA: Kanamycin, VA: Vancomycin, †: Reference strain *Staphylococcus aureus* NCTC 12989, **: Susceptibility tests were performed on two randomly selected isolates from each type of samples for both CoPS and CoNS

Table 6: Antibiotic susceptibility of 7 coagulase-positive staphylococci isolated from the nasal mucosa of food handlers in Makkah, Saudi Arabia

| | R | IR | S |
|---|---|---|---|
| | N (%) | N (%) | N (%) |
| OX | 3 (43) | 0 | 4 (57) |
| VA | 7 (100) | 0 | 0 |
| KA | 0 | 0 | 7 (100) |
| PG | 7 (100) | 0 | 0 |
| CO | 5 (71.4) | 0 | 2 (28.6) |
| RP | 0 | 0 | 7 (100) |
| E | 0 | 0 | 7 (100) |

R: Resistant, IR: Intermediate resistance, S: Sensitive, E: Erythromycin, RP: Rifampicin, CO: Colistin sulphate, PG: Penecillin G, OX: Oxacillin, KA: Kanamycin, VA: Vancomycin

Table 7: Antibiotic susceptibility of 12 coagulase-negative staphylococci isolated from the nasal mucosa of food handlers in Makkah, Saudi Arabia

| | R | IR | S |
|---|---|---|---|
| | N (%) | N (%) | N (%) |
| OX | 2 (16.6) | 0 | 10 (83.4) |
| VA | 5 (41.7) | 0 | 7 (58.3) |
| KA | 0 | 0 | 12 (100) |
| PG | 12 (100) | 0 | 0 |
| CO | 3 (25) | 0 | 9 (75) |
| RP | 0 | 0 | 12 (100) |
| E | 1 (8.3) | 1 (8.3) | 10 (83.3) |

S: Sensitive, IR: Intermediate resistance, R: Resistant, E: Erythromycin, RP: Rifampicin, CO: Colistin sulphate, PG: Penecillin G, OX: Oxacillin, KA: Kanamycin, VA: Vancomycin

All CoPS isolates that were recovered from the nasal mucosa of food handlers, only 43% of the isolates (n = 7) were resistant to oxacillin, however, all seven isolates (100%) showed resistance to both vancomycin and penicillin G (Table 6). Resistance patterns of twelve CoNS isolates from the nares of food handlers were 16.6% to oxacillin, 41.7% to vancomycin, 100% to penicillin G, 25% to colistin sulphate and 8.3% to erythromycin. Only one CoNS isolate (8.3%) was resistant to four out of the seven antibiotics used (Table 7).

## DISCUSSION

staphylococci are ubiquitous bacteria found on the skin and mucous membranes of humans and warm-blooded animals. They also can be recovered from different environmental sources such as soil and water and from wide range of foodstuffs (Irlinger, 2008).

Of all nine ready-to-drink raw milk examined in this survey, 88.9% were positive for staphylococci (Table 1). The prevalence of staphylococci was found to be significantly higher ($p = 0.001$) in bovine raw milk with mean colony counts of $5.7\times10^3$ cfu $mL^{-1}$ (Table 2). The presence of high numbers of staphylococci in raw bovine and camel milk that was found in this study (Table 1) may indicates that the lactating animals suffer from *Staphylococcus*-associated mastitis (Peles *et al*., 2007). This may be supported by the detection of CoPS in all bovine and camel raw milk samples (Table 4). *Staphylococcus aureus* (CoPS) is the causative agent of mastitis in lactating animals (Bartolomeoli *et al*., 2009). Similar to our findings, high levels of *Staph. aureus* were found in raw camel milk in Qassim region, central Saudi Arabia (El-Ziney and Al-Turki, 2007), in bovine milk in Hungary and Algeria (Peles *et al*., 2007; Ghazi *et al*., 2010). Other possible sources that contribute to high levels of *Staph. aureus* in raw milk is the improper hygiene and poor farm management (Ateba *et al*., 2010). In contrast to bovine and camel raw milk, the prevalence of staphylococci (Table 1), particularly CoPS (Table 3) in raw caprine milk was remarkably low. This may indicates the practice of proper hygiene measures and/or pasteurization. In general, high levels of CoPS in raw milk (>$10^5$ cfu $mL^{-1}$) may constitute public health concern and since staphylococci can grow in raw milk stored at 10-40°C for several hours (Do Carmo *et al*., 2002), the consumption of ready-to-drink raw milk examined in this study should be avoided.

The total absence of staphylococci in processed cheese (Table 1) may be due to the heat treatment and the proper hygiene practice during the production process. All Egyptian-imported romy cheese (yellow) and Hungarian-imported feta cheese (white) yielded staphylococci (Table 1). The significantly ($p = 0.005$) highest levels of staphylococci found in Egyptian-imported romy cheese (Table 3) and the presence of CoPS (Table 4) may suggest pasteurization failure or contamination after pasteurization due to poor hygiene practice (Rosengren *et al*., 2010). In many countries, low-degree contamination of cheese by *Staphylococcus aureus* (e.g., up to $10^3$ cfu $g^{-1}$) is not considered to be a risk for public health (Kluytmans, 2010). In Hungarian-imported feta cheese, no CoPS were detected (Table 3) and the low levels of CoNS that were found (Table 1) suggests a very low safety hazards (Irlinger, 2008; Even *et al*., 2010), since CoNS are frequently isolated from a wide range of foodstuffs, particularly ripened fermented cheese (Coton *et al*., 2010).

The presence of *Staphylococci* in different types of water has been reported (Harakeh *et al*., 2006; Faria *et al*., 2009). In the city of Makkah, water tankers with different capacities (e.g., 29 and 39 $cm^3$) usually supply drinking water to households' private reservoirs on demand. Poor conditions of these tankers can result in poor quality of water. In this survey, very low prevalence (20%, n =10) of staphylococci was observed in drinking water (Table 1) that was sampled directly from different water tankers with different capacities. Mihdhdir (2009) investigated the microbiological water quality of drinking water supplied by water tankers in

Makkah and reported the presence of *Staph. aureus* in about 25% of the samples. In contrast with Mihdhdir (2009) study, no CoPS were detected from drinking water in this study (Table 3). Similar to our results, Faria *et al.* (2009) reported low numbers of CoNS ($10^0$-$10^2$ cfu 100 $mL^{-1}$) in drinking water in Portugal. It is important to note that staphylococci are considered to be one of the genera that commonly found in drinking water as Heterotrophic Plate Count (HPC) bacteria. It was suggested that it is not possible to establish health-based standard for HPC bacteria in drinking water (Allen *et al.*, 2004).

In the current survey, we investigated the presence of staphylococci in potable water-related biofilm, in household taps. A total of nine taps from three different houses were swabbed and another nine different taps with filters from the same three houses were swabbed. Taps with filters have been used for more than 7 months without any cleaning/changing of filters. Of the nine taps swab, only 55.6% were positive for staphylococci (Table 1), with one sample only (2.0%) positive for CoPS (Table 4). With regards to tap-filters swab, the incidence of staphylococci was slightly higher (66.7%, n = 9) (Table 1), with only two samples (33.3%) yielded CoPS (Table 4). Coagulase-negative staphylococci are well known for their ability to produce biofilm formation on different surfaces, which considered as one of the main virulence factors for CoNS implicated in nosocomial infections (Piette and Verschraegen, 2009). Both CoPS and CoNS were found to form biofilm on the surface of water line pipes in dental clinics (Lancellotti *et al.*, 2007) and on stainless steel pipes in milk processing plant (Michu *et al.*, 2011). The presence of staphylococci (Table 1), including CoPS (Table 4) in biofilm in drinking water distribution system reported in this study may cause aesthetic and hygienic problems as HPC bacteria in biofilm formations can inherit resistance to disinfectants and their long term persistence can deteriorate the microbiological quality of water. Furthermore, potential waterborne pathogen may take refuge within biofilm formation and persist for long periods, with the possibility of acquiring resistance to antimicrobial agents due to transferable resistance genes (Lee and Kim, 2003; Parsek and Singh, 2003).

The mucous membranes of the human nasopharynx and animal skin are the main ecological niches for staphylococci (Irlinger, 2008). Thus, it is not surprising that all nasal swabs of food handlers that were examined in this survey were positive for the presence of staphylococci (Table 1). However, the nasal carriage rates of *Staphylococcus aureus* (CoPS) by healthy adult population was reported to range between 20 and 55% (Acco *et al.*, 2003; Wertheim *et al.*, 2005; Mainous *et al.*, 2006; Kluytmans, 2010). Food handlers play significant role in food safety, particularly in the transmission of food borne *Staph. aureus* (Al-Turki *et al.*, 1998). The risk of contamination increases when food is being manipulated without proper training of personnel in good manufacturing and handling practice (Acco *et al.*, 2003). In this survey the nasal colonization of *Staph. aureus* in food handlers was found to be high (Table 4), this observation is in agreement with the results reported in other studies (Hatakka *et al.*, 2000; Acco *et al.*, 2003) regardless of variations in sample size.

Antibiotic resistance is a major public health concern since resistant bacteria can persist and circulate in the environment with possible transmission to humans via contaminated food and water. Consequently, methicillin-resistant *Staphylococcus aureus* (MRSA) is currently the most commonly identified antibiotic-resistant pathogen in many countries worldwide including Saudi Arabia (Bukhari and Al-Otaibi, 2009; Bukharie, 2010; Ippolito *et al.*, 2010). In the present study we found that CoPS that were isolated from all types of raw milk, romy cheese and biofilm were resistant to penicillin G and oxacillin (Table 5). This is expected since *Staphylococcus aureus*, particularly MRSA is well-known for its remarkable resistance against all **β**-lactams

(Normanno *et al*., 2007b). Moreover, CoPS strains that derived from raw camel milk, romy cheese and biofilm exhibited resistance to vancomycin (Table 5). This is probably because MRSA stains that are resistant to beta-lactam drugs may develop induced resistance to vancomycin (Deresinski, 2007; Ateba *et al*., 2010). The multidrug-resistance of CoPS found in raw milk (Table 5) may be explained by the over use of antibiotic treatment in veterinary medicine in Saudi Arabia. The incidence of beta-lactams and/or vancomycin resistant *Staphylococcus aureus* in raw milk, cheese and potable water-related biofilm has been reported worldwide (Lancellotti *et al*., 2007; Bartolomeoli *et al*., 2009; Ateba *et al*., 2010). In Saudi Arabia, however, available information on the incidence of multidrug-resistant *Staph. aureus* in food and the environment are sparse, thus to the best of our knowledge this is the first isolation of oxacillin and vancomycin-resistant *Staphylococcus aureus* from dairy products and potable water-related biofilm in western Saudi Arabia.

Of all *Staphylococcus aureus* isolates that were recovered from the nares of food handlers, 43 and 100% were resistant to oxacillin and penicillin G, respectively (Table 6). Moreover, a remarkable level of resistance to vancomycin (100%, n = 7) was also observed (Table 6). Similar results with respect to penicillin G were reported by Tondo *et al*. (2000) among *Staph. aureus* recovered from personnel of a dairy product factory in Brazil. In Saudi Arabia, much attention has given to multidrug resistant CoPS in clinical sittings, thus the nasal carriage of multidrug-resistant MRSA had been reported among hospitalized patients and healthcare personnel (Khalil and Al-Ruaily, 2008; Ahmad, 2010). In contrast to present study, most of the multidrug-resistant MRSA that were recovered from the nares of healthy adults were found to exhibit full susceptibility to vancomycin (Al-ghaithy *et al*., 2000; Acco *et al*., 2003; Khalil and Al-Ruaily, 2008; Akhi *et al*., 2008; Ahmad, 2010). On the other hand, other researchers reported the isolation of vancomycin-resistant *Staphylococcus aureus* (VRSA) from clinical specimens other than nasal swabs (Mehdinejad *et al*., 2008; Al-Obeid *et al*., 2010). The possible explanation of this discrepancy is the influence of various technical factors on the detection of resistance to glycopeptides in clinical laboratories (Deresinski, 2007; Piette and Verschraegen, 2009). In general, the high levels of multidrug-resistance among CoPS of various origins reported in this survey (Table 5 and 6) are not surprising in a region with unrestricted availability of antibiotics in clinical and veterinary medicine (Al-ghaithy *et al*., 2000; Al-Obeid *et al*., 2010).

It is worth mentioning that all *Staphylococcus aureus* isolates examined in this survey from all types of samples were susceptible to erythromycin, rifampicin and kanamycin (Table 5 and 6). To date, it is well established that multidrug-resistant MRSA and VRSA remain susceptible to a number of antimicrobial agents, such as rifampicin (Casey *et al*., 2007). Therefore, it was suggested that a combination of glycopeptides and rifampicin and/or tetracycline may be considered as first-line option for the treatment of serious MRSA infections (Casey *et al*., 2007; Deresinski, 2009).

The coagulase-negative Staphylococci may tend to be more resistant to antibiotics than *Staph. aureus* and easily develop multiresistance (Taponen and Pyorala, 2009). The results reported in this study are in accordance with that statement (Table 5 and 7). Indeed, CoNS derived from camel raw milk were remarkably resistant to five out of seven antibiotics used (erythromycin, colistin sulphate, penicillin G, oxacillin and vancomycin), whereas CoPS from the same type of sample showed resistance to three antimicrobials (penicillin G, oxacillin and vancomycin) (Table 5). All CoNS isolated from dairy products, water and biofilm samples in this survey showed full resistance to penicillin G and oxacillin (Table 5). Resistance to penicillin G was also noted among all CoNS of nasal origin, with only May 4, 201116.6% (n = 12) were resistant to oxacillin

(Table 7). Resistance to penicillin G and methicillin/oxacillin in CoNS of dairy products, potable water, biofilm and clinical origins have been reported worldwide (Gooraninejad *et al.*, 2007; Lancellotti *et al.*, 2007; Moniri *et al.*, 2007; Resch *et al.*, 2008; Mehdinejad *et al.*, 2008; Faria *et al.*, 2009; Sawant *et al.*, 2009; Even *et al.*, 2010). The resistance to penicillin G in CoNS is explained by the production of β-lactamase (Taponen and Pyorala, 2009), while resistance to methicillin in CoNS is encoded by *mec*A gene which is located on *Staphylococcus* Cassette Chromosome (SCC). The SCC can be transferred horizontally between various staphylococcal species which suggests that CoNS act as reservoir for the dissemination of resistance genes to CoPS (Irlinger, 2008; Piette and Verschraegen, 2009). Resistance to erythromycin and vancomycin was also observed among CoNS in this survey (Table 5 and 7). Resistance to erythromycin is increasing progressively among CoNS isolates worldwide and CoNS were the first bacterial species to acquires resistance to glycopeptides (e.g. vancomycin) (Piette and Verschraegen, 2009). It is worthwhile mentioning that food has been considered as a potential reservoir of vancomycin-resistant bacteria (Gazzola and Cocconcelli, 2008) including CoNS (Table 5). Thus, multidrug-resistance in CoNS should be considered as a major public health concern.

The consumption of dairy products containing CoNS (e.g., feta cheese, Table 1) is considered to be safe, since to date, no available reports of CoNS-associated health hazards following the ingestion of dairy products (Irlinger, 2008; Coton *et al.*, 2010; Even *et al.*, 2010). Nevertheless, the occurrence of multidrug-resistant CoNS in dairy products (Table 5) and the possible transfer of drug-resistance between microorganisms in food matrices, suggests that only specifically selected, antibiotic susceptible strains of CoNS should be used in starter cultures (Irlinger, 2008; Resch *et al.*, 2008; Even *et al.*, 2010). In the same way, the CoNS in drinking water may be a minor component of the whole bacterial communities (Faria *et al.*, 2009), though, the presence of multidrug-resistance CoNS strains (Table 5) and their remarkable ability to produce biofilm formation in drinking water distribution system, may represent public health hazards (Lancellotti *et al.*, 2007; Faria *et al.*, 2009). It has been suggested that the regulation of biofilm formation and antibiotic resistance in CoNS seem to use similar pathways, i.e., multidrug-resistance can be significantly higher in biofilm positive strains compared to biofilm-negative ones (He *et al.*, 2009; Piette and Verschraegen, 2009). To the best of our knowledge, we reported the first isolation of multidrug-resistant coagulase-negative staphylococci from dairy products, drinking water, drinking water distribution system (biofilm) and human mucous membrane in western Saudi Arabia. Additional studies are needed to investigate the prevalence of antibiotic-resistant CoNS in food and clinical samples in Saudi Arabia to assess their possible public health hazards.

## CONCLUSION

The current survey may provide an insight on the wide spread of multidrug-resistant strains of *Staphylococcus aureus* and coagulase-negative staphylococci in food and the environments of western Saudi Arabia. The results reported in this survey may explain, in part, the increasing incidence of community-associated MRSA infections in Makkah and probably highlight for the first time the possible public health risks associated with the wide spread of multidrug-resistant CoNS in food and the environment in Saudi Arabia. Given the emergence of CoNS as potential nosocomial pathogens worldwide, their ability to develop multiresistance and to transfer resistance genes to other species, the results of this study suggest that public health authorities in Saudi Arabia should pay attention to the presence of antibiotic-resistant CoNS in human samples and their relationship to contact with animals or environmental contamination.

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