Detection of antibiotic resistant *Staphylococcus aureus* among male carriers in Jeddah Cites

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Abstract: Different bacterial species such as *Pseudomonas* sp., *E. coli* and *Staphylococcus* sp. were isolated from fingernails, nasal cavity and saliva of 70 workers of different nationalities in Jeddah. *Staphylococcus aureus* was identified by API system and biochemical testing. The occurrence percentage of *Staphylococcus aureus* in nasal cavity, saliva and fingernails samples were ranged between 23.72 to 72.32%, 19 to 94.49% and 11.36 to 92.54%, respectively. The occurrence percentage of *S. aureus* was high in a few workers at 28°C and humidity of 28 or 57 RH%, but at higher temperature (32°C) and humidity (44%) it was increased in nasal cavity of all workers in one restaurant. Concerning sensitivity of *S. aureus* to antibiotic, the results showed that 40 isolates were sensitive to Cefotaxime and Cefoxitin, 11 isolates were sensitive to all antibiotics, 9 isolates were non- multi drug resistant (NMDR) but they were resistant to one antibiotic only, and 50% of isolates were multi drug resistant (MDR). It was also clear that two isolates from nasal and fingernails were resistant to seven antibiotics and two isolates from fingernails were resistant to 14-15 antibiotics.
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1. Introduction

Humans are the main source of the microorganisms, which are found in about 30% to 50% of the population. *Staphylococci* are widespread in nature. They can be found in the air, in dust, in water, and on humans and animals. The main human reservoirs of these organisms are the skin and nasal cavity, Jay, (1986). The *Staphylococcus* genus includes at least forty species. Of these; nine have two subspecies and one has three subspecies, Harris and Foster, (2002). Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. They are found worldwide, they are a small component of soil microbial flora, Madigan and Martinko(2005). Staphylococcal food-borne diseases are estimated to cause 6–81 million illnesses and up to 9000 deaths, and accounts for 14–20% of outbreaks involving contaminated food in the USA , Mead *et al.*, (1999).

Staphylococci can be present in the throat, nasal area and also under the fingernails as commensal inhabitants. *Staphylococcus aureus* is a common cause of food intoxication. An estimated 50% of adults are *S. aureus* carriers with colonization commonly occurring in/on the nose, Jay (1992). Contaminated hands are a major source of cross-contamination in the food service kitchen ,Bean *et al., (*1997); Colombari *et al.,* (2007). Strains present in the nose often contaminate the back of hands, fingers and face and so, nasal carriers can easily become skin carriers. Although it is difficult to determine the origin of the strains involved in Staphylococcal food poisoning outbreaks, food

handlers are usually regarded as one of the primary source of these organisms, Genigeorgis, (1989). It is generally accepted that hands are an important vehicle of food cross-contamination and that improved personal hygiene and scrupulous hand washing would lead to the basic control of feces-to-hand to- mouth spread of potentially pathogenic transient microorganisms, Allwood *et al.,* (2004); Sneed *et al*., (2004) and Strohbehn *et al.,* (2008).

Staphylococcus aureus is found in the nostrils, on the skin and hair of warm-blooded animals. Up to 30-50% of the human population are carriers. *Staphylococcus aureus* is able to grow in a wide range of temperatures from 7 to 48.5°C with an optimum of 30 to 37°C, Schmitt *et al.,* (1990), pH from 4.2 to 9.3, with an optimum of 7 to 7.5, Bergdoll, (1989) and sodium chloride concentrations up to 15% NaCl. These characteristics enable *S. aureus* to grow in a wide variety of foods.

Staphylococcus aureus can cause localized and invasive infections in humans. This is attributed to its ability to produce a variety of virulence factors such as capsular polysaccharides, staphylococcal enterotoxins (SEs), toxic shock syndrome toxin 1 (TSST-1) O'Riordan and Lee (2004). Clinical signs range from minor skin conditions (e.g., pimples, boils and impetigo) to more severe disease such as cellulitis and postoperative wound infections. *S. aureus* can also cause pneumonia, bacteremia, meningitis, sepsis, and pericarditis. *S. aureus* can also cause food poisoning and toxic shock syndrome (Community-Associated MRSA Information for Clinicians, 2008). Therefore, it is important to detect

S. aureus carriage Most of the studies on *S. aureus* associated with food poisoning have focused on screening of the isolates for enterotoxins, Cha *et al*., (2006). Al-Bustan *et al*., (1996); Figueroa *et al., (* 2002) with only sparse data on the carriage of other virulence factors and antimicrobial resistance among *S. aureus* obtained from food handlers, Udo *et al.,* (1999); Loeto *et al.,* (2007). The global spread of multidrug-resistant bacteria has increased in the past decade, due to the increased mobility of human populations, Kunin, (1993).

The aim of the present study is to isolate *S. aureus* from nasal cavity, saliva and fingernails of workers in Jeddah city. Also, to study the prevalence of *S. aureus* susceptibility and resistance to antibiotics.

2.Materials and Methods
2.1. Materials:
2.1.1. Sample of the study:
The study was conducted on swabs from nasal cavity, saliva and fingernails of 70 adult male of different nationalities in Jeddah City from 2008 to 2009.

2.1.2. Media used:
-Tryptic soy agar(bio Merieux, Hazelwood, MO) + 5% sheep blood
- Nutrient agar (NA, Oxoid CM3)

2.1.3. Kits:
API system of identification (Analytical Profile Index, BioMerieux, Durham, NC, USA), chart to determine the bacterial code which was compared to the (API) 20E Codebook for accurate identification of the organism.

2.2. Methods:
2.2.1. Collection and isolation of bacteria from food handlers
Sterile culture swabs (Becton-Dickinson, Sparks, MD) was used in order to obtain samples. Each swab was moistened with 0.9% NaCl (w/v) and then rubbed across a pre-determined surface area. It was collected from nasal cavity, saliva and under the finger nails, then introduced into tubes. After sampling, each swab was stored in a holder containing a moistened sponge (provided by Becton-Dickinson) and analyzed within 3 h.

2.2.2. Isolation of *Staphylococcus aureus*
Each sample was streaked on the surface of a tryptic soy agar + 5% sheep blood plate. After 48 hr incubation at 35°C, the plates were examined for hemolytic colonies. Each hemolytic colony type was re-striked onto a tryptic soy agar + 5% sheep blood plate, incubated for 48 hr at 35°C. Then, cell

morphology and Gram reaction were recorded. Bacterial slants of nutrient agar (as stock culture) were prepared for further biochemical testing.

In addition, BHI broth tubes (1ml in each) were inoculated each with one of the colonies grown on the nutrient agar slants and incubated for 24 hours at 37ºC.

2.2.2. Bacterial identification and characterization:
Bacteria were identified as described by Beumer *et al*. (1996). Colonies were observed for size, texture, color and hemolytic reactions. Tests for coagulase, DNase producers, anaerobic fermentation of glucose and manitol, and hemolysin production using sheep blood were carried out according to Lancette and Tatini (1992).

Further identification of enteric organisms was done using the API 20E system. Colonies from BAP were harvested and mixed with 0.5 ml McFarland standard until turbidity of the solution and a bacterial suspension was obtained. Using a sterile pipette, the bacterial suspension was inoculated to rehydrate each of the wells making sure that the end of the pipette touched the end of the cupule, allowing capillary action to draw the fluid into the well as bulb was slowly squeezed. Inoculation of specific test wells was done according to the manufacturer's instructions. The strips were incubated for 18 to 24 h at 37°C. Test results were logged to the API system for complete identification.

2.2.3. Sensitivity to antibiotics
Forty identified *S. auerus* isolates were subjected to sensitivity testing using 26 antimicrobial agents, Kloos and Banerman (1999). They were determined by vetek2 compact (bioMérieux Corporate) and *S. aureus* ATCC 29213 was used for quality.

3. Results and Discussion
The study was performed by analyzing of seventy adult male workers from different nationalities in Jeddah City.

Several bacterial species were isolated from their fingernails, nasal cavity and saliva such as *Pseudomonas* sp., *E. coli* and *Staphylococcus* sp. The occurrence percentage of *Staphylococcus* sp. was ranged between 23.72 to 72.32%, 12.25 to 94.54% and 11.36-92.04% from nasal cavity, saliva and fingernails, respectively. These results supported the findings of Evangelista-Barreto and Vieira (2003) who observed that approximately 60% of 24 fish handlers from two fishmongers were carriers of *S. aureus* either on their hands, nasal cavities or saliva.

The characteristics of *Staphylococcus* cells in most samples were Gram-positive, immobile, in

irregular collection, DNase producers, catalase and coagulase-positive strains and possessed the ability to glucose fermentation. Strains showed clot formation (coagulase positive) within 2 h.; 75, 81.48 and 87.1% of them formed firm clots after 6, 12 and 24 h respectively at 35ºC. Our results were in agreement with that obtained by Kloos and Lambe (1991) and Martin and Myers (1994) who reported that the percentage of coagulase-positive strains increased with increasing incubation time. However, Chang and Huang (1996) found that the sensitivity of the coagulase test was 98.1% after 6 h of incubation.

Results showed that most of the 70 workers tested were contaminated with *S. aureus*. The percentage of *S. aureus* in nasal cavity, saliva and fingernails were ranged between 0.0 to 72.32%, 0.0 to 94.54% and 0.0 to 92.04%, respectively. The occurrence percentage of *S. aureus* was very high which indicated that hygiene was not the first priority in many of their life style. Borges *et al,.* (2011) demonstrated that hygienic practices may be overlooked. These poor hygienic practices could facilitate the transmission of bacteria to different places, where they could contaminate equipment, utensils and food with which they come into contact.

Table (1) showed the maximum percentages of workers contaminated with *S. aureus* in nasal cavity (31.43 and 54.29%), saliva (42.86 and 41.43%) and fingernails (34.29 and 45.71%) were observed when the percentage of *S. aureus* was ranged between 20 to 40% and 40 to 60%, respectively. When the percentage of *S. aureus* was ranged between 80 to 100% the workers were only contaminated in saliva (4.29%) and fingernails (1.43%). High frequency of carrier status among the workers has been identified by several investigators and many investigation studies conducted on Staphylococci carrier status in human in many countries, including Brazil (3, 4, 6, 8, 17, 18, 20 and 21). Results in Table (2) clarified that the maximum percentage of *S. aureus* occurrence in nostrils (21.36 -70.00%) and fingernails (21.36 - 85.59%) was observed at 28ºC and 57 R.T. At the same time, *S. aureus* in fingernails recorded higher percentage (0.00 - 92.04%) at higher temperature (35ºC) and humidity (35 R.T). However, the highest percentage of *S. aureus* in saliva (80.70 – 94.5% and 0.00-94.54%) was recorded at 32ºC, 44 R.T and 35ºC, 35 R.T, respectively.

About 40 *S. aureus* were isolated from them to study their sensitivity to antibiotics. The results investigated that there were variations between isolates in their response to antibiotics (Table 3). Some types were found to be resistant to some antibiotics and sensitive to others. Table (3) revealed that *S. aureus* isolates were found to be more

resistant to ampicillin (32.5%) and penicillin G (35%). The lower resistant *S. aureus* isolates recorded 2.5% with gentamicin, meropenem and 5% with amoxicillin, clavulaanate and oxacillin. All isolates appeared to be highly sensitive to cefotaxime and cefoxitin (100%). In this study there was no isolates similar to the control isolate (*S. aureus* ATCC29213).

Table 1. The percentage of contamination with *S. aureus* in nasal cavity, saliva and fingernails at different levels of contamination

S. aureus %	% Workers contaminated with *S. aureus*		
	Nasal Cavity	**Saliva**	**Finger Nails**
00-20	2.86	5.71	10.00
20-40	31.43	42.86	34.29
40-60	54.29	41.43	45.71
60-80	14.29	5.71	8.57
80-100	0.00	4.29	1.43

Table 2. The effect of temperature and humidity on the percentage of *S. aureus* among workers tested

Temp. (⁰C)	Humidity (R.H)	% *S. aureus*		
		Nasal Cavity	**Saliva**	**Finger-nails**
28	26	29.76 - 64.34	16.12 - 59.79	11.16 - 60.71
28	57	21.36 - 70.00	24.39 - 60.59	21.36 - 85.59
32	44	69.03 - 69.4	80.70 - 94.54	00.00
35	35	25.68 - 61.29	00.00 - 94.54	00.00 - 92.04
38	26	19.00 - 39.93	25.39 - 70.80	28.49 - 47.64

Distribution of non-multidrug resistant (NMDR) and multidrug resistant (MDR) *S. aureus* phenotypes revealed that 27.5% isolates were sensitive to all antibiotics, 22.5% were resistant to one antibiotic (NMDR) and 50% were MRD (Table 4). These results are important because MDR *S. aureus* strains pose a threat to common antibiotic treatment for routine infections. The widespread presence of antibiotic resistant microorganisms and highlights the importance of good hygiene practices in the fight against antibiotic resistant infectious agents.

As obvious from Table 4, one isolate from fingernails of different carriers was resistant to four antibiotics, 3 isolates from nasal cavity, saliva and fingernails were resistant to six antibiotics, two isolates from nasal cavity and fingernails were resistant to seven antibiotics and two isolates from fingernails were resistant to 14 and 15 different antibiotics.

MDR strains were defined as being resistant to two or more chemicals from different antimicrobial classes, Rabatsky-Her *et al*., (2004).

Table 3. Sensitivity of 40 *S. aureus* isolates to antibiotics strainsAntibiotics

	Resistant isolates			Total resistant isolates (%)	% Total sensitive isolates (%)
	Nasal Cavity	Saliva	Fingers & Nails		
Amoxicillin, Clavulaanate	0	0	1	5.00	95.0
Ampicillin	7	4	2	32.5	67.5
Cefotaxime	0	0	0	0.00	100
Cefoxitin	0	0	0	0.00	100
Ceftriaxone	2	2	0	10.0	90.0
Ciprofloxacin	1	1	1	7.50	92.5
Clarithromycn	1	1	4	15.0	85.0
Clindamycin	3	1	2	15.0	85.0
Erythromycin	2	1	2	12.5	87.5
Gentamicin	1	0	0	2.50	97.5
Linezolid	0	0	2	5.00	95.0
Meropenem	1	0	0	2.50	97.5
Nitrofurantoin	1	0	3	10.0	90.0
Oxacillin	0	0	2	5.00	95.0
Penicillin G	6	3	5	35.0	48.15
Rifampin	0	0	3	7.50	92.5
Teicoplanin	2	2	6	25.0	75.0
Tetracycline	0	0	2	5.00	95.0
Vancomycin	1	0	2	7.50	92.5
Azithr0mycin	0	0	2	5.00	95.0
Clarithromych	0	0	2	5.00	95.0
Fosfomycin	1	1	4	15.0	85.0
Beta-Lactamace	0	1	1	5.00	95.0
Norfloxacin	0	0	2	5.00	95.0
Fusidic Acid	1	1	2	10.0	90.0
Mupiocin	1	0	4	15.0	85.0

Table 4. Percentage of non-multidrug resistance (NMDR) and multidrug resistance (MDR) 40 *S. aureus* isolated from food handlers

No. of Antibiotics		No. of resistant strains	Multi-drug resistant strains (%)
Sensitive	0	11	27.5
NMDR	1	9	22.5
MDR	2	8	20.0
	3	4	10.0
	4	1	2.50
	6	3	7.50
	7	2	5.00
	14	1	2.50
	(15)	1	2.50

Borges *et al.,* (2011) reported that resistance to penicillin G (66.7%) was the most common resistance pattern with 10 isolates. Erythromycin was the next most common resistance: with nine isolates (60.0%) displayed resistance, and six isolates (40.0%) showed intermediate sensitivity. They found four isolates (26.7%) were resistant to tetracycline. Additionally, all *S. aureus* isolates were sensitive to oxacillin, vancomycin, ciprofloxacin and gentamicin. Martins *et al.* (2007) recorded that 100% of *S. aureus* strains isolated from enteral diet and handler samples were resistant to tetracycline and 90% were resistant to erythromycin. Udo *et al.* (2009) found in total 185 (92.5%) of the 200 isolates expressed resistance to antibacterial agents. They were resistant to penicillin G (82.0%), tetracycline (19.0%), erythromycin (2.5%), clindamycin (2.0%), trimethoprim (7.5%), kanamycin (2.5%), streptomycin (1.5%),

ciprofloxacin (1.5%), fusidic acid (1.0%) and cadmium acetate (68.0%). Seventy-six (38.0%) and 114 (57.0%) isolates had type 5 and type 8 capsular polysaccharides, respectively.

Resistance to a specific drug is often a part of larger package of resistance factors located on plasmids or transposons, Alterthum (2004). Penicillin was originally found to be extremely effective in treating *S. aureus* infections, but penicillin-resistant strains of *S. aureus*, mediated by the production of β-lactamase (an enzyme that inactivates the lactam ring of β-lactam antibiotics), began to develop. Methicillin was introduced in 1959 to treat human patients with staphylococcal infections resistant to penicillin. According to Tavares (2000), the resistance to antibiotics, is explained not only by the presence of resistance genes but also by expression of these genes, which is controlled by the environment.

As workers represented a section of the healthy population in the community, besides working in restaurants, the detection of high prevalence of antibiotic resistance in *S. aureus* isolated from them also highlights the growing problem of antibiotic resistance in the community (Udo *et al.,* 2009). This increased potential for resistance in the population of susceptible isolates should be considered in monitoring actions to reduce resistance to antibiotics in the food chain (Klein and Bulte 2003). The spread of resistant microorganisms by food and/or food handlers is worrisome and should be avoided in the production chain (Borges *et al*., 2011).

Although in some countries individuals colonized with *Staphylococci* are not allowed to handle food, this is not a practical solution to the problem, because it is difficult to control. The best solution is the proper training of food handlers in order to prevent the contamination of vulnerable foods, and to instruct them on the need of proper storage of such foods. It is important that hands be washed properly and obeying health instructions (MontvIle, *et al.,* 2001).

Conclusion

This study has provided data on the carriage of *Staphylococcus aureus*, and initial information on the prevalence of antibacterial resistance in *S. aureus* obtained from a random sample of workers from different nationalities in Jeddah, Saudi Arabia. Our results should be contributed to better management of *S. aureus* carriers specially if these workers are among the food handlers in order to enhance the safety of restaurant customers.

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