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Original Article

Screening of Nasal and Hands Carriage of Methicillin-Resistant Staphylococci Colonization among Lebanese Nutraceuticals Handlers

Nada El Darra[1], Karim Raafat[2,*], Iman El-Ghazzawi[1]

ABSTRACT

Introduction: Poor personal hygiene of the nutraceuticals handlers could be a potential source of diseases due to the high occurrence of bacterial contamination. **Methods:** A cross sectionnal study was conducted among Lebanese nutraceuticals handlers. A pretested structured questionnaire was used for collecting information on age, sex, marital status, service years, educational status, status of training of nutraceuticals-handlers. **Results:** Nasal and hands swabs were investigated for S. *aureus*, MRSA, CoNS and MRSE.Among 144 nutraceuticals handlers, 41% and 69% exhibited no bacterial growth in their nasal and hand swabs.The carrier rate of S. *aureus* was 11.8 and 5.6% for nose and hands in nutraceuticals handlers.The nasal and hands carrier rate of MRSA is 6.3% and 2.1%, respectively.MRSE presented a rate of 10.4% and 7.6% for the nasal and hands carriage. **Conclusion**: The findings showed that the Lebanese nutraceuticals handlers are a vehicle of different resistant bacterial strains in their nasal and hands carriage,which poses a significant risk to the Lebanese consumer.

Key words: Nutraceuticals handlers, S.*aureus*, Resistant, Nasal, Hands, Carriage.

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INTRODUCTION

Nutraceuticals-borne diseases caused by microbiological agents represent a major health problem especially in developing countries such as Lebanon, where nutraceuticals safety scandals are a serious concern. Outbreaks and sporadic cases of nutraceuticalsborne disease are of increasing occurrence in Lebanon and in response to that, the Ministry of Public Health have done a series of inspections and publicized a list of restaurants, supermarkets and other nutraceuticals provider services that did not meet the ministry's nutraceuticals regulation standards.[1] Poor nutraceuticals handling practices and poor hygiene status among nutraceuticals handlers were among the major risk factors in the transmission of bacteria and contamination of nutraceuticals thus posing serious health risks to consumers.[2-4] According to Gizaw *et al* (2013), almost 75% of nutraceuticals borne illness outbreaks is related to improper nutraceuticals handling practices in nutraceuticals establishments.[5]

Consequently, the microbiological quality of nutraceuticals handlers' hands from nutraceuticalsservice operations should be maintained in a good condition to reduce the existence of multidrug resistance bacteria.[6]

Among the bacterial strains contaminating nutraceuticals, *Staphylococci* have a recordof developing resistance quickly and successfully to antibiotics. This definitive response is a consequence of acquisition and transfer of antibiotic resistance genes.[7] The two major microorganims in the Staphylococcus genus are Staphylococcus *aureus* and Staphylococcus *epidermis*.

Staphylococcus aureus (S. *aureus*) has long been recognized asone of the most important agent of nutraceuticals poisoning worldwide. The primary habitat of *S. aureus* is themucous membranes of the human nasopharynx.[8] Staphylococcus *aureus* is associated with several disease processes including skin and soft tissue infections, and pneumonia and can be a major public health concern.[9] Furthermore, the consumption of nutraceuticalss containing *Staphylococcus aureus* can cause severe gastro-intestinal illness.[10] The contamination of nutraceuticals with *S. aureus* could be due to improper manipulation by the nutraceuticals handlers, who are frequently contaminated with this bacteria.[11]

Over the past decade, an increase of methicillin-resistant S. *aureus*(MRSA) colonization and infection has become particularly important to global health care.[10] MRSA has been present in various types of nutraceuticals including pork, beef ,bovine milk and cheese.[12-14].The existence of multidrug resistant bacteria may pose seriousthreat to the consumers, hence, the microbiological quality on nutraceuticals handlers' hands should be maintained in a good condition byadhering to hygienic standards reducing the existence of multidrugresistance bacteria.[6]

Staphylococcus *epidermidis* (S.*epidermidis*) is the most frequently isolatedmember of the group of coagulase-negative staphylococci. It colonizes the skin and mucousmembranes of the human body and represents the major partof the normal bacterial flora of this habitat. However, it has gained a substantial interest in recent years, as a human pathogen, due to

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its abilityto form biofilms on indwelling medical devices and artificial prothesis.[15] These biofilms are considered as one of the virulence factors of this bacteria, which is easily transmissible in the community.[16]

The emergence of mecithillin resistant staphylococci strains , due to the presence of mec A gene[17] has become a burning issue nowaday.[18]

To our knowledge, this is the first study undertaken to determine the hands and nose colonization of MRSA and MRSE among Lebanese nutraceuticals handlers. The main objective of this study is to detect the nasal and hand colonization of Staphylococcus *aureus* (S. *aureus*) and coagulase negative staphylococci (CoNS) seeking for methicillin-resistant staphylococcus *aureus* (MRSA) and methicillin-resistant staphylococcus epidermidis (MRSE) among a randomly selected sample ofLebanese nutraceuticals handlers. A pretested structured questionnaire was used for collectinginformation on age, sex, marital status, serviceyears, educational status, status of training and habits ofhand washing of each nutraceuticals-handler.

MATERIALS AND METHODS

Study design and area

A cross sectional study was conducted among nutraceuticals handlers working in different nutraceuticalsservice establishments including cafeterias, coffee shops and restaurants surrounding Beirut Arab University from 24 January 2017 till July 2017.

Sample size and sampling procedure

The sample include One hundred forty-four nutraceuticals handlers who were randomly selected from the nutraceuticalsservice establishments surrounding the university.

Data collection procedure.

The questionnaire used in this study were validated by Dagnew *et al.*, 2012.[19] This questionnaire include information on age, sex, marital status, service years, educational status, training status and different hygienic practices for each nutraceuticals handler. Nasal and Hands swabs were collected aseptically from each nutraceuticals handler.

Microbiological procedure

From each enrollee, two sterile swabscontaining transport medium (Meus s.r.l. Via L.da vinci 24/b, Piove di Sacco, Italy) were collected. One nasal swab, the other is a hand swab collected at the palm of the hands as well as under the nail and interdigital. The labelled swabs were transferred to the biomedical laboratory of the Faculty of Health Sciences, for microbiology investigation, within half an hour.

Nasal and hand swabs from each enrollee were cultured on plates of mannitol salt agar (Oxoid, CM0085) and incubated overnight at 37°C. After the proper incubation period, the plates were examined for the presence of yellow and pink colonies, presumably S. *aureus*and CoNS, respectively. The different colonies were subjected to gram stained films catalase test and coagulase test (slide and tube) to prove the presence of S. *aureus*or CoNS.[20] Then, each of the previous identified strains was subjected to cefoxitin sensitivity test. The test was performed according to CLSI recommendation for disc diffusion using 30 µg cefoxitin disc (Oxoid, 30 µg).[21] From each strain, 0.5 MacFarland was prepared in saline and swabbed onto plates of Muller hinton agar (Oxoid, CM0337) (each strain was swabbed on half-plate), the cefoxitin disc was then placed in the center of the half plate. All plates were incubated for overnight at 37°C.

Interpretation of the cefoxitin sensitivity test

The zone of inhibition was measured including the disc, then the results were recorded. If the zone of inhibition is 21 mm or less for S.*aureus*, it is recorded as MRSA.. If the zone of inhibition is 24 mm or less for CoNS, it is recorded as MRSE.

Statistical analysis

All data were entered, cleaned and analyzed using SPSS 21 (SPSS Inc. Chicago, II, USA). Statiscal significance was accepted at $p< 0.05$.

Ethical consideration

The data was collected after a signed written consent form obtained from all the nutraceuticals handlers participating in this study. This study was approved (IRB number 2016H-0026-HS-R-0182) by the Research Ethics Committee of Beirut Arab University.

RESULTS AND DISCUSSION

Sociodemographic characteristics

A total of 144 nutraceuticals handlers (129 males and 15 females) participated in this study. Table 1 shows the sociodemographic characteristics of the different nutraceuticals handlers, including the

Table 1: Sociodemographic characteristics of the nutraceutical's handlers Z test for column proportions.

Characteristics	Female n= 15	Male n = 129	Total n= 144	Chi square P value
Age in years	**No (%)**	**No (%)**	**No (%)**	**X^2 = 14.664 P value = 0.26**
18-28	4 (26.7)[a]	77 (59.7)[b]	81 (56.3)	
29-39	9 (60)[a]	26 (20.2)[b]	35 (24.3)	
40-49	0 (0)[a]	14 (10.9)[a]	14 (9.7)	
50-59	2 (13.3)[a]	10 (7.8)[a]	12 (8.3)	
60+	0 (0)[a]	2 (1.6)[a]	2 (1.4)	
Marital Status				X^2 = 19.703 P value = 0.0001
Single	7 (46.7)[a]	79 (61.2)[b]	86 (59.7)	
Married	8 (53.3)[a]	50 (38.8)[a]	58 (40.3)	
Education Status				X^2=0.392 P value = 0.942
Illiterate	1 (6.7)[a]	5 (3.9)[a]	6 (4.2)	
Grade 1-8	5 (33.3)[a]	40 (31)[a]	45 (31.3)	
Grade 9-12	7 (46.7)[a]	62 (48.1)[a]	69 (47.9)	
Certificate	2 (13.3)[a]	22 (17.1)[a]	24 (16.7)	
Training on nutraceuticals safety				X^2=1.388 P value = 0.708
No	13 (86.7)[a]	104 (80.6)[a]	117 (81.3)	
Basic training	1 (6.7)[a]	20 (15.5)[a]	21 (14.6)	
Advanced training	1 (6.7)[a]	4 (3.1)[a]	5 (3.5)	
Serving years in the Nutraceuticals service establishment				X^2=6.630 P value = 0.085
< 1 year	8 (53.3)[a]	33 (25.6)[b]	41 (28.5)	
1-10 years	7 (46.7)[a]	72 (55.8)[a]	79 (54.9)	
11-20 years	0 (0)[a]	13 (10.1)[a]	13 (9)	
21+	0 (0)[a]	11 (8.5)[a]	11 (7.6)	
Holding a health certificate				X^2=5.530 P value = 0.019
Yes	13 (86.7)[a]	71 (55)[b]	84 (58.3)	
No	2 (13.3)[a]	58 (45)[b]	60 (41.7)	
Total	**15 (10.4)**	**129 (89.6)**	**144 (100)**	

educational levels, age category, sex and work experiences. The ages of the participants ranged between 18 and 60, with the highest percentage for nutraceuticals handlers aging between 18-28 (56.3%). With respect to the marital status, 60% of them were single and 40% married. Approximately half (50 %) of the nutraceuticals handlers had a middle school education, with (35.5%) did not have a middle school education (less than 8 years of education or illiterate). Regarding the serving years in the nutraceuticals service establishment, 41 (28.5 %), have less than 1 year of experience. Almost, the majority 117 (81.3%) of the participants did not attend any training on nutraceuticals safety. Out of the 144 nutraceuticals handlers, 60 (41.7%) do not hold a health certificate.

Hygienic practices assessment of the nutraceuticals handlers

The assessment of the hand washing practices of nutraceuticals handlers shows that the majority of the nutraceuticals handlers, 143 (99.3%) have the habit of hand washing with water and soap after toilet, except one nutraceuticals handler. While 5 (3.5 %) and 6 (4.2 %) of the nutraceuticals handlers do not wash their hands, before and after preparing nutraceuticals, respectively.Regarding having pets (cats and dogs) or poultry (chicken) at home, 12 (8.3 %) and 4 (2.8 %) have pets and poultry, respectively at home.Out of the 144 nutraceuticals handlers, 33 (22.9 %) took antibiotics during the last three weeks, which could be a hint of developing resistance bacterial strains. The majority of the nutraceuticals handlers, 136 (94.4 %) were not been admitted to hospital in the last three months. Table 2 shows that 131 (91%) of the participants do not suffer from any skin infection or gastrointestinal disturbance.

Microbiological results of nose and hands growth

Nasal and hands swab cultures of 144 nutraceuticals handlers have been investigated for the presence for S.*aureus*, MRSA, CoNS and MRSE. The nose and hand carriage of the differet bacterial strains is shown in Table 3. S. *aureus* were isolated from 17 (11.8%) of the nasal swabs and 8 (5.6%) hand swabs of the personnel investigated. The rate of isolation of S.*aureus* from the nasal culture in our study is less than the rate found by Dagnew *et al.*, 2012, who showed 20.5% of S.*aureus* from the nasal cultures.[19] However, our results were found to be higher than the rate of 0.77% obtained from a study conducted on Nutraceuticals handlers in Turkey.[22] Hatakka et al., showed as well a high prevalene of S.*aureus* in nasal samples of flight-catering staff.[12] Higher S. *aureus* prevalence among nutraceuticals handlers, of 44.6%, 53.2% and 23.1% was noted in Botswana, Kuwait, and South-eastern Anatolia, respectively.[23] All these studies have highlighted the high potential of contaminating nutraceuticals with S.*aureus*, resulting consequently in severe infections. The prevalence of nasal and hand carriers of MRSA was 9 (6.3 %) and 3 (2.1 %) respectively. Our findings were found to be lower than the ones of Kasturwar *et al.*, 2011, who showed a high prevalence of MRSA with a percentage of 30% among nasal swabs of nutraceuticals handlers. Our results showed that the prevalence of CoNS in nose (30.6%) and hands (16%) was higher than the prevalence of S.*aureus*, with respectively a percentage of 11.8% and 5.6% in nose and hands.The prevalence of nasal and hand carriers of MRSE was 15 (10.4 %) and 11 (7.6 %) respectively.

Sociodemographic in relation to nasal and hands carriage of S.aureus,MRSA, CoNS and MRSE

Table 4 represents the sociodemographic in relation to nasal and hands carriage of S.*aureus*.Among the 144 nutraceuticals handlers, the lowest rate of nose colonization with S.*aureus* (11.8%), MRSA (11.1%) , CoNS (18.2%) and MRSE (26.7%) were obtained in the educational status of certificate. Similar results were obtained for hands colonization. Thus, reflecting that the high level of education may increase hygiene awareness and decrease the bacterial contamination. Table 4 shows the highest rate of colonization for nasal and hands swabs for the nutraceuticals handlers not receiving any nutraceuticals safety training with the following percentages S.*aureus* (94.1%, 100%), MRSA (7.6%, 100%) , CoNS (86.4%, 78.3%) and MRSE (80%. 81.8%). Among the 5 nutraceuticals handlers receiving advance training, only 1 presented at the nasal carriagne MRSE. These results demonstrated the importance of providing training to nutraceuticals handlers, since the majority of the ones holding the different bacterial strains in their nasal and hand carriage lack proper training in nutraceuticals handling operations, mass feeding and sanitary practices.[24] The lowest rate of colonization were obtained for the nutraceuticals handlers serving between 10 to 21 years, with the following percentages for nasal and hands carriage, S.*aureus* (17.7%, 25%), MRSA (22.2%, 0%) , CoNS (15.9%, 7.7%) and MRSE (20%, 36.4%). Higher rate were observed for the nutraceuticals handlers serving less than 1o years with the following percentages, for nasal

Table 2: Hygienic practices of the nutraceuticals handlers.

Variables	No (%)
Hand washing after going to the toilet by water	
Yes	143 (99.3)
No	1 (0.7)
Hand washing after going to the toilet by soap	
Yes	143 (99.3)
No	1 (0.7)
Hand washing before preparing nutraceuticals	
Yes	139 (96.5)
No	5 (3.5)
Hand washing after preparing nutraceuticals	
Yes	138 (95.8)
No	6 (4.2)
Having pets (cats and dogs) at home	
Yes	12 (8.3)
No	132 (91.7)
Having living poultry (chicken) at home	
Yes	4 (2.8)
No	140 (97.2)
Taking antibiotics during the last three weeks	
Yes	33 (22.9)
No	111 (77.1)
Being admitted to hospital in the last three months	
Yes	8 (5.6)
No	136 (94.4)
Suffering from any skin infection	
Yes	13 (9)
No	131 (91)
Suffering from gastrointestinal disturbance	
Yes	13 (9)
No	131 (91)
Total	144 (100)

Table 3: Microbiological results of Nose and Hands growth.

Microbiological result	Nose growth n = 144	Hands growth n = 144
No growth	59 (41)	99 (68.8)
S. *aureus*	17 (11.8)	8 (5.6)
MRSA	9 (6.3)	3 (2.1)
CoNS	44 (30.6)	23 (16)
MRSE	15 (10.4)	11 (7.6)
Total	144 (100)	144 (100)

Table 4. Sociodemographic in relation to nasal and hands carriage of S.*aureus*, MRSA, CoNS and MRSE

	Nose Growth					Hands growth				
Characteristics	**No growth**	**S. aureus**	**MRSA**	**CoNS**	**MRSE**	**No growth**	**S. aureus**	**MRSA**	**CoNS**	**MRSE**
Education Status										
Illiterate	1 (1.7) [a]	1 (5.9) [a]	1 (11.1) [a]	3 (6.8) [a]	0 (0) [a]	3 (3) [a]	0 (0) [a]	1 (33.3) [b]	1 (4.3) [a,b]	1 (9.1) [a,b]
Grade 1-8	20 (33.9) [a]	6 (35.3) [a]	2 (22.2) [a]	11 (25) [a]	6 (40) [a]	30 (30.3) [a]	3 (37.5) [a]	0 (0) [a]	7(30.4) [a]	5 (45.5) [a]
Grade 9-12	29 (49.2) [a]	8 (47.1) [a]	5 (55.6) [a]	22(50) [a]	5 (33.3) [a]	49 (49.5) [a]	4 (50) [a]	2 (66.7) [a]	11 (47.8) [a]	3 (27.3) [a]
Certificate	9 (15.3) [a]	2 (11.8) [a]	1 (11.1) [a]	8 (18.2) [a]	4 (26.7) [a]	17 (17.2) [a]	1 (12.5) [a]	0 (0) [a]	4 (17.4) [a]	2 (18.2) [a]
Training on nutraceuticals safety										
No	43 (72.9) [a]	16 (94.1) [a]	9 (7.6) [a]	38 (86.4) [a]	12 (80) [a]	80 (80.8)	8 (100) [a]	3 (100) [a]	18 (78.3) [a]	9 (81.8) [a]
Basic training	12 (20.3) [a]	1 (5.9) [a]	0 (0) [a]	6 (13.6) [a]	2 (13.3) [a]	14 (14.1) [a]	0 (0) [a]	0 (0) [a]	5 (21.7) [a]	2 (18.2) [a]
Advanced training	4 (6.8) [a]	0 (0) [a]	0 (0) [a]	0 (0) [a]	1 (6.7) [a]	5 (5.1) [a]	0 (0) [a]	0 (0) [a]	0 (0) [a]	0 (0) [a]
Serving years in the Nutraceuticals service establishment										
< 1 year	17 (28.8) [a]	3 (17.6) [a]	2 (22.2) [a]	15 (34.1) [a]	4 (26.7) [a]	27 (27.3) [a]	3 (37.5) [a]	1 (33.3) [a]	7 (30.4) [a]	3 (27.3) [a]
1-10 years	33 (55.9) [a]	11 (64.7) [a]	5 (55.6) [a]	22 (50) [a]	8 (53.3) [a]	55 (55.6) [a]	3 (37.5) [a]	2 (66.7) [a]	15 (65.2) [a]	4 (36.4) [a]
11-20 years	2 (3.4) [a]	2 (11.8) [a, b]	0 (0) [a,b]	6 (13.6) [a,b]	3 (20) [b]	7 (7.1) [a]	2 (25) [a,b]	0 (0) [a,b]	1 (7.7) [a, b]	3 (27.3) [b]
21+	7 (11.9) [a,b]	1 (5.9) [a,b]	2 (22.2) [b]	1 (2.3)	0 (0) [a, b]	10 (10.1) [a]	0 (0) [a]	0 (0) [a]	0 (0) [a]	1 (9.1) [a]
Holding a health certificate										
Yes	30 (50.8) [a]	11 (64.7) [a,b]	8 (88.9) [b]	26 (59.1) [a,b]	9 (60) [a, b]	43 (43.4) [a]	2 (25) [a]	1 (33.3) [a]	11 (47.8) [a]	3 (27.3) [a]
No	29 (49.2) [a]	6 (35.3) [a,b]	1 (11.1) [b]	18 (40.9) [a,b]	6 (40) [a, b]	56 (56.6) [a]	6 (75) [a]	2 (66.7) [a]	12 (52.2) [a]	8 (72.7) [a]

and hands carriage, S.*aureus* (85.7%, 75%), MRSA (82.3%, 100%) , CoNS (84.1%, 92.3%) and MRSE (80%, 65.6%).Our results indicate the importance of earning experience to reduce the contamination level in different bacterial strains.

There is no statistical siginificance between holding a health certificate and the presence of the different bacterial strains.

CONCLUSION

Nutraceuticals handlers play an important role in nutraceuticals safety and can contribute in several nutraceuticals borne diseases, since thet may introduce pathogens during nutraceuticals processing. This study indicate that the Lebanese nutraceuticals handlers may be a potential source of different resistant bacteria strains, which can spread consequently nutraceuticals borne diseases, resulting in a significant risk for the Lebanese consumers. The government should impose the implementation of nutraceuticals safety training for nutraceuticals handlers as well as a continuous monitoring of their personal hygiene.

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AUTHORS CONTRIBUTION

All authors contributed equally to this research.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

ABBREVIATIONS

MRSA; methicillin-resistant *S. aureus,* S.*epidermidis*; Staphylococcus *epidermidis;* S. *Aureus*; Staphylococcus *aureus*, CoNS; coagulase negative staphylococci seeking for methicillin-resistant staphylococcus *aureus*, and MRSE; methicillin-resistant staphylococcus epidermidis.

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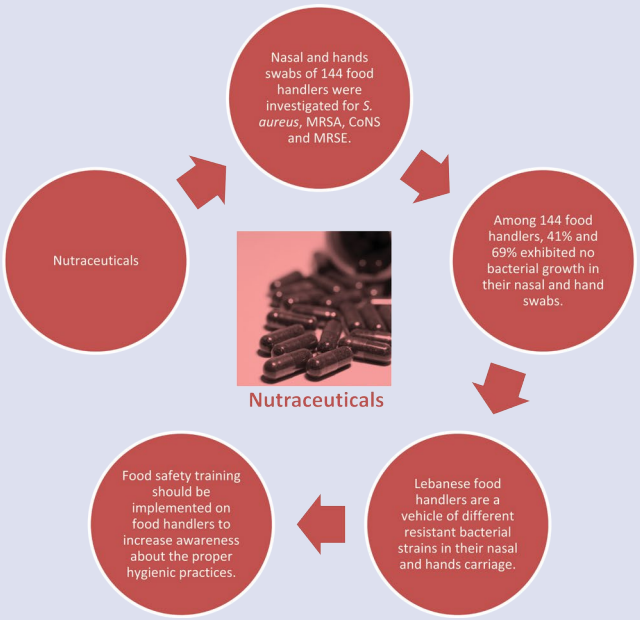
GRAPHICAL ABSTRACT



SUMMARY

Nasal and hands swabs of 144 food handlers were investigated for *S. aureus*, MRSA, CoNS and MRSE. Among 144 food handlers, 41% and 69% exhibited no bacterial growth in their nasal and hand swabs. Lebanese food handlers are a vehicle of different resistant bacterial strains in their nasal and hands carriage. Thus, food safety training should be implemented on food handlers to increase awareness about the proper hygienic practices.

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