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Nasal carriage rate and antimicrobial resistance pattern of *Staphylococcus aureus* among the food handlers in Canton Sarajevo, Bosnia and Herzegovina

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ABSTRACT

Introduction: The nasals and hand carriage of *Staphylococcus aureus* in food handlers (FHs) represent a significant source of Staphylococcal food contamination and food poisoning. Antimicrobial resistance (AMR) is a microorganism's ability to resist the action of one or more antimicrobial agents. *S. aureus* has demonstrated the ability to rapidly respond to each new antimicrobial with the development of a resistance mechanism. The aim of the study was to assess the prevalence of nasal carriage rate and AMR pattern of isolated strains *S. aureus* among FHs in Canton Sarajevo, Bosnia and Herzegovina.

Methods: The retrospective study included laboratory results of 11.139 tested subjects between January 2014 and December 2018. The study was conducted in the laboratory of the Institute of Public Health of the Federation of Bosnia and Herzegovina in Sarajevo. Samples of nasal swabs were collected from FHs, employees in companies located in Canton Sarajevo, during sanitary surveillance prescribed by applicable legal standards. *S. aureus* isolates were identified according to conventional microbiological methods and antimicrobial susceptibility testing was performed by the agar disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing; 2013 standard.

Results: Among the 11.138 subjects, 792 (7.1%) were carriers of *S. aureus.* Isolated strains were tested on eight different antibiotics, and the resistance to penicillin, ampicillin, and amoxicillin was 788 (99.5%), 776 (97.9%), and 752 (94.9%), retrospectively. In total, 86.36% of isolated strains were multidrug-resistant.

Conclusions: The low percentage of *S. aureus* carriers indicates that preventive measures of carrier control are being actively implemented within the legally prescribed measures. The emergence of numerous isolated strains with multidrug-resistance characteristics is a significant public health problem and consequently limits the range of antibiotics available for therapeutic purposes. The results of this research indicate that AMR has increased in Sarajevo Canton and it is following the trend of global growth.

Keywords: Antimicrobial drug resistance; food handlers; *Staphylococcus aureus* carriage

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INTRODUCTION

Staphylococcus aureus represents the most common pathogen in the Staphylococcaceae family (1). It is a Gram-positive coccus, non-motile, and does not form spores. When viewed through a standard

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microscope, it is arranged in grapes or irregular clusters (2). A classical approach to *S. aureus* identification includes coagulase and catalase tests. Incorrect species identification can negatively impact on effective treatment and control measures (3). Isolated colonies on agar are round, convex, 1-4 mm in diameter, and with a sharp border. On blood agar plates, colonies are frequently surrounded by zones of clear beta hemolysis. *S. aureus* can grow in a broad range of temperatures (7-48.5°C; optimum 30-37°C), pH (4.2-9.3; optimum 7-7.5), and up to 15% NaCl (4). The golden appearance of colonies of some strains represents the etymological root of the bacteria's name *aureus* (4) and the pigment has been linked to virulence (5,6).

S. aureus can cause various infections and it is considered as an important nosocomial pathogen. As an opportunistic pathogen, it frequently asymptomatically colonizes the anterior nares of humans and animals. Nasal carriage of *S. aureus* can be permanent or intermittent and may build the reservoir for autogenous infections and spreading to other individuals (7). Close to 20-30% of healthy people are carriers of this specific microorganism (8).

The nasal and hand carriage of *S. aureus* in food handlers (FHs) represent a significant source of Staphylococcal food contamination and Staphylococcal food poisoning (SFP), because of its capability to produce toxins in food (9-11). Although several Staphylococcal enterotoxins have been identified, highly heat-stable toxin A, remain the most primary cause of SFP worldwide (12). Therefore, it is crucial to reliably detect *S. aureus* carriage among FHs (13). Bosnian national legislation is in line with the World Health Organization (WHO) recommendations, which underline extremely important to have regularly scheduled check-ups so those infected employees involved in direct contact with food can be immediately excluded and appropriately treated (14,15). At present, in Bosnia and Herzegovina, screening for *S. aureus* carriage is mandatory for all food-producing companies twice during the year (14). Conferring on to the European Food Safety Authority (EFSA), in 2017, 20 states in Europe reported food-borne outbreaks caused by bacterial toxins, including Staphylococcal (16). During 2016 and 2017, Bosnia and Herzegovina reported three outbreaks with 30 human cases. The contributory factor in common was cross-contamination by an infected FH (17,18). Several studies reported food poisoning to be a result of infection with enterotoxigenic strains of *S. aureus*, mostly during poultry outbreaks (19-22).

Antimicrobial resistance (AMR) has become one of the greatest threats to global health. It represents a microorganism's ability to resist the action of one or more antimicrobial agents. Development of AMR is a typical phenomenon caused by mutations in bacterial genes, and many of the genetic elements coding for AMR can also spread between bacteria. In addition, a bacterium can acquire several resistance mechanisms and hence be resistant to several antimicrobial agents, which is extremely problematic as it may severely limit the available treatment alternatives for the infection (23). *S. aureus* demonstrated the ability to rapidly respond to each new antimicrobial with the development of a resistance mechanism, starting with penicillin and methicillin (24). Today, *S. aureus* has become resistant to many commonly used antibiotics. The aim of the study was to assess the prevalence of nasal carriage rate and AMR pattern of isolated strains *S. aureus* among FHs in Canton Sarajevo, Bosnia and Herzegovina.

METHODS

The retrospective study included laboratory results of 11.139 subjects, tested between January 2014 and December 2018. The study was conducted in the laboratory of the Institute of Public Health of the Federation of Bosnia and Herzegovina in Sarajevo.

Sample collection

Samples of nasal swabs were collected from FHs, employees in companies located in Canton Sarajevo, during sanitary surveillance prescribed by applicable legal standards (14). Sampling was performed during the early morning hours, mostly in the laboratory of the institute. For companies with numerous employees, sampling was organized as a part of field activities, and these samples were immediately forwarded for bacteriological analysis in the laboratory. The nasal specimen was collected using swab moistened with sterile saline solution. The tips were inserted 1-2 cm inside both nostrils and rotated six times. Next, the swabs were inserted into sterilized screw-capped tubes.

S. aureus isolation and identification

The nasal swab specimens were inoculated on a 5% blood agar plate. After incubation for 24 hours at 37°C under aerobic conditions, the plates were examined. Isolates were identified by conventional methods, including Gram staining, colony morphology, hemolysis, biochemical tests, tests for catalase, and coagulase activity (25). Catalase and coagulase-positive Staphylococcal isolates were identified by the API STAPH system (bioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

Antimicrobial susceptibility testing of the *S. aureus* isolates

The testing was performed by the agar disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) (26) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (27). All tests were performed on Mueller-Hinton agar. The bacterial suspension having visually equivalent turbidity to 0.5 McFarland standards was prepared. The surface of agar was then lightly and uniformly inoculated and incubated at 37°C for 18-24 hours. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler and classified as sensitive (S), intermediate (I), and resistant (R). The results for penicillin G (10 IU), ciprofloxacin (5 μg), erythromycin (15 μg), and gentamicin (10 μg) were interpreted according to the EUCAST (27), while fusidic acid, ampicillin (10 μg), and cefotaxime were interpreted according to the CLSI criteria (26,28). As a quality control, the control strain of *S. aureus* ATCC 25923 was used.

In this study, multidrug resistance (MDR) was considered as AMR of isolated strain to three or more antimicrobial classes (29). Beta-lactams were represented with penicillin (penicillin G, ampicillin, and amoxicillin) and cephalosporins (cefotaxime) and other classes with above-mentioned antibiotics. Methicillin-resistant *S. aureus* prevalence could not be determined because the standard antibiotic set at the time of the study did not include cefoxitin.

Statistical analysis

Observed values are presented in the form of tables. The distribution of subjects through the year is described as frequency and percentage. Testing for antibiotic resistance variation is conducted using a one-way analysis of variance (ANOVA) testing with *post hoc* multiple comparisons, with the initial year's resistance being used as the control value. The data were analyzed with IBM SPSS Statistic 25.00 (IBM Corporation, Armonk, New York) and MS-Excel. Statistical significance was established with $p < 0.05$. The results of resistance were compared through years with Dunnett's *t*-test, which treat one group as a control and compare all other groups against it.

RESULTS

Analysis of results showed that of a total of 11.139 FHs, 792 (7.1%) were colonized by *S. aureus*. As presented in Table 1, the lowest number of carriers was registered during 2018, in total 121 (5.1%), and the highest in 2015, a total of 202 (8.8%). There is also a noticeable increase in the number of tested FHs at an annual level.

In the present study, resistance patterns of 792 isolates collected for 5 years from the nares of FHs showed overall high levels of resistance. The resistance rate was 96.1-100% ampicillin and 83.8-100% to amoxicillin. During the 5 years, there was also an increase in resistance to erythromycin and gentamicin from 72.2% to 96.8% and 94.3% to 99.2%, retrospective. The increase was notable for all tested antibiotics, with statistical significance $p = 0.000$, except penicillin ($p = 0.426$), according to results of one-way ANOVA testing, as shown in Table 2.

TABLE 1. Nasal carriage rate of *S. aureus* among food handlers, in Canton Sarajevo, 2014-2018 year

Year	Positive		Negative		Total	
	n	%	n	%	n	%
2014	155	7.5	1.911	92.5	2.066	100
2015	202	8.8	2.090	91.2	2.292	100
2016	146	6.3	2.142	93.7	2.286	100
2017	168	7.9	1.957	92.1	2.125	100
2018	121	5.1	2.249	94.9	2.370	100
Total	792	7.1	10.349	92.9	11.139	100

S. aureus: Staphylococcus aureus

TABLE 2. Antimicrobial resistance pattern of *S. aureus* isolated from food handlers in Canton Sarajevo, 2014-2018 year (one-way ANOVA testing)

Antibiotic	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	Mean ± SD	F	p
Penicillin G	98.7	99	100	100	100	99.54±0.64	1.06	0.426
Ampicillin	96.1	95	100	100	100	98.22±2.47	26.18	0.000
Amoxicillin	83.8	92.6	100	100	100	95.28±7.17	245.63	0.000
Cefotaxime	1.3	22.8	22.2	36.3	42.2	24.96±15.79	590.06	0.000
Ciprofloxacin	9	14.4	17.4	21.4	19.8	14.60±8.58	274.782	0.000
Erythromycin	72.2	86.1	89.6	89.9	96.8	86.92±9.10	245.93	0.000
Gentamicin	94.3	88.6	98.6	96.4	99.2	95.42±4.28	61.915	0.000
Fusidic acid	18.1	13.4	18.1	25	29.8	20.88±6.48	140.27	0.000
					Total	66.98±39.05	69.87	0.000

S. aureus: Staphylococcus aureus, SD: Standard deviation, ANOVA: Analysis of variance

For Dunnett's *t*-test, the initial 2014 was taken for reference value as the year with the lowest value of the antibiotic resistance is observed and, at the same time, the starting year in a perceived period. Based on the results, the *p*-value for all antibiotics in the period 2014-2018 was 0.000, except for penicillin ($p = 0.014$). The increase of resistance to fusidic acid is significant in 3 years ($p = 0.000$), except in 2016 ($p = 1.000$). Table 3 presents the results of multiple comparisons of resistance for all tested antibiotics throughout the years.

A total of 684 isolated strains of *S. aureus* were resistant at three or more classes of antibiotics, and it is considered to represent multidrug resistance. Among them, 327 were resistant to three, 293 to four, and 63 to five classes of antibiotics. Just one isolated strain *S. aureus* from 2016 was resistant to all six classes, as shown in Table 4.

DISCUSSION

Studies from numerous countries indicated that FHs were one of the significant sources of food contamination (8,13). Since FHs carriers of *S. aureus* may contaminate the food and cause SFP outbreak, this study was undertaken to assess the prevalence of carriers and AMR patterns of isolated strains, among FHs in Sarajevo Canton. Our study revealed that about 7.1% were carriers of *S. aureus*. Only one study was conducted in the territory of Bosnia and Herzegovina in the area of Zenica-Doboj Canton. According to Uzunovic results, *S. aureus* was isolated in 189 (1.4%) subjects (30). The low percentage of *S. aureus* carriers in our study is correlated with EFSA reports of three outbreaks with 30 human cases during 2 years in our country (17,18). Only in the study by Gündüz et al. conducted in Turkey among a similar population, a lower number is reported (0.77%) (31). In studies conducted in Europe, values ranged from 20 to 24.7% (32,33), with a significant difference compared to undeveloped countries 57.5%, 65%, and 71.8%, in retrospect (10,34,35). Contradicted results may reflect variation in numerous populations living in different geographical regions and at the same time, workers work with various foods (meat, dairy products, vegetables, grains, etc.). Supporting this theory Ho et al. reported that colonization rates were considerably higher in workers handling raw meats (30%) than in non-exposed workers (13%) (36). Furthermore, differences in results could be based on various factors such as personal hygiene habits of workers, level of education, hygiene of utensils-equipment, and the environment the FHs are working in and the regulations on the inquest (36-38). We consider that the education of FHs is crucial in preventing bacterial carriage. We do not have accurate data on course attendance, although we can say with great assurance that this preventive measure is being implemented. According to our results, the incidence of *S. aureus* carriers among FHs is low, but the disadvantage of the study due to its design is the impossibility of detection of enterotoxins by the Enzyme-linked Immunosorbent Assay/polymerase chain reaction.

Since the discovery, penicillin has been effective in treating the *S. aureus* until the bacteria have become

TABLE 3. Multiple comparisons of resistance through the years (Dunnett's t two-sided)

Dependent variable	(I) Year	(J) Year	Mean difference (I-J)	Standard error	Significant	95% confidence interval	
						Lower bound	Upper bound
Penicillin	2015	2014	0.30000*	0.08165	0.014	0.0640	0.5360
	2016	2014	1.30000*	0.08165	0.000	1.0640	1,5360
	2017	2014	1.30000*	0.08165	0.000	1.0640	1.5360
	2018	2014	1.30000*	0.08165	0.000	1.0640	1.5360
Ampicillin	2015	2014	−1.10000*	0.08165	0.000	−1.3360	−0.8640
	2016	2014	3.90000*	0.08165	0.000	3.6640	4.1360
	2017	2014	3.90000*	0.08165	0.000	3.6640	4.1360
	2018	2014	3.90000*	0.08165	0.000	3.6640	4.1360
Amoxicillin	2015	2014	8.80000*	0.08165	0.000	8.5640	9.0360
	2016	2014	16.20000*	0.08165	0.000	15.9640	16.4360
	2017	2014	16.20000*	0.08165	0.000	15.9640	16.4360
	2018	2014	16.20000*	0.08165	0.000	15.9640	16.4360
Cefotaxime	2015	2014	21.50000*	0.08165	0.000	21.2640	21.7360
	2016	2014	20.90000*	0.08165	0.000	20.6640	21.1360
	2017	2014	35.00000*	0.08165	0.000	34.7640	35.2360
	2018	2014	40.90000*	0.08165	0.000	40.6640	41.1360
Ciprofloxacin	2015	2014	14.39997*	0.08165	0.000	14.1640	14.6360
	2016	2014	17.39997*	0.08165	0.000	17.1640	17.6360
	2017	2014	21.39997*	0.08165	0.000	21.1640	21.6360
	2018	2014	19.79997*	0.08165	0.000	19.5640	20.0360
Erythromycin	2015	2014	13.90000*	0.08165	0.000	13.6640	14.1360
	2016	2014	17.40000*	0.08165	0.000	17.1640	17.6360
	2017	2014	17.70000*	0.08165	0.000	17.4640	17.9360
	2018	2014	24.60000*	0.08165	0.000	24.3640	24.8360
Gentamicin	2015	2014	−5.70000*	0.08165	0.000	−5.9360	−5.4640
	2016	2014	4.30000*	0.08165	0.000	4.0640	4.5360
	2017	2014	2.10000*	0.08165	0.000	1.8640	2.3360
	2018	2014	4.90000*	0.08165	0.000	4.6640	5.1360
Fusidic acid	2015	2014	−4.70000*	0.08165	0.000	−4.9360	−4.4640
	2016	2014	0.00000	0.08165	1.000	−0.2360	0.2360
	2017	2014	6.90000*	0.08165	0.000	6.6640	7.1360
	2018	2014	11.70000*	0.08165	0.000	11.4640	11.9360

*The mean difference is significant at the 0.05 level

TABLE 4. Multidrug resistance pattern of *S. aureus* isolated from food handlers in Canton Sarajevo, 2014-2018 year

Year	Resistance to classes of tested antibiotics									
	Three[a]		Four[b]		Five[c]		Six[d]		Total	
	n	%	n	%	n	%	n	%	n	%
2014	78	75.73	23	22.33	2	1.94	0	0	103	100
2015	98	57.88	58	34.32	13	7.69	0	0	169	100
2016	63	49.22	53	41.41	11	8.59	1	0.78	128	100
2017	55	33.33	92	55.76	18	10.91	0	0	165	100
2018	33	27.73	67	56.30	19	15.97	0	0	119	100
Total	327	47.81	293	42.84	64	9.21	1	0.15	684	100

[a]Penicillins, cephalosporin, fluoroquinolone; [b]a+macrolide; [c]b+ aminoglycoside; [d]c+fusidic acid. *S. aureus: Staphylococcus aureus*

resistant. Throughout the second half of the 20th century, new antibiotics such as methicillin and vancomycin were developed, which successfully treated *S. aureus* infections (39). Our study demonstrates that almost all of the nasals isolates of *S. aureus* 788 (99.5%), 776 (97.9%) and 752 (94.9%) were resistant to penicillin, ampicillin, and amoxicillin, respectively. Since 1943, when penicillin was first introduced, *S. aureus* was resistant to the drug in 5-7% of cases (40). According to Hughes et al., in the late '70s, an average of 85% of isolates was penicillin-resistant (41). Our results show high primary resistance to isolated strains of *S. aureus* on penicillin, ampicillin, and amoxicillin, and over the past 3 years, it has risen to 100%. Rebic et al. presented identical results for outpatients in the Sarajevo Canton during 2015 (42). Our results are correlated with the Emeakaroha et al. study in Nigeria (43). A notable increase in penicillin resistance is also reported in the United Kingdom, where only 2% of *S. aureus* isolates are antibiotic sensitive (44). According to the results of a study in Ethiopia, resistance to penicillin and ampicillin was 51.2%, 46.3%, and 90.7%, respectively (45,46). The frequencies of resistance to specific agents were 61.3% for penicillin and ampicillin in Spain (21). In Kuwait, the resistance is 82% (47). This significant rate of resistance among staphylococci strains can be ascribed, in most cases, to the production of beta-lactamase enzyme that destroys the beta-lactam ring and inactivates the penicillin antibiotic; this enzyme is also encoded by the plasmid which makes it easier to be transferred among strains, or other genetic elements (48).

The frequencies of resistance to specific antibiotics in our study were 25.4% for cefotaxime, 14.6% for ciprofloxacin, 86.5% for erythromycin, 95.8% for gentamicin, and 20.3% for fusidic acid. Our results of resistance of isolated *S. aureus* strains to erythromycin and gentamicin differ significantly from the results of studies in the world, according to which erythromycin resistance ranged from 2.5 to 25.8% (21,36,45-47) and gentamicin 3.5% and 6.5%, respectively (21,46). In a study by Bektas et al. conducted in the Sarajevo Canton among outpatients up to 5 years, 61.5% of *S. aureus* strains were resistant (49). Furthermore, ciprofloxacin resistance (14.6%) in our study is lower compared to the Beyene et al. study conducted in Ethiopia (46), but significantly higher than the study of Udo et al. in Kuwait (1.5% and 1%, respectively, for ciprofloxacin and fusidic acid) (47).

According to the Centers for Disease Control and Prevention (CDC), by the '70s community-acquired isolates of *S. aureus* often were resistant to penicillin, whereas nosocomial strains typically were resistant to multiple antibiotics. By the end of the 1970s, the antimicrobial rates for both types of strains were practically equal (50). Our results support the CDC's view that they are nosocomial strains, but because of the inability to implement molecular methods, it is impossible to determine the origin and mechanism of the resistance, while at the same time supporting WHO report released April 2014 stated, "this serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country. Antibiotic resistance – when bacteria change so antibiotics no longer work in people who need them to treat infections is now a major threat to public health" (40). A total of eight different antibiotics were tested within the study, and among isolated strains of *S. aureus*, 86.36% were found to possess multidrug resistance (resistance against three or more different classes of antibiotics). Our results are correlated with a study by Alghaithy et al. conducted in Saudi Arabia, which also confirmed significantly higher multidrug resistance (89%) in strains isolated from outpatients (51). According to studies by Argudín et al. and Beyene et al. conducted in Ethiopia and Spain, multidrug resistance was confirmed in 16.3% and 19.4% of isolated strains of *S. aureus*, respectively (21,46). In the Argudín et al. study conducted among FHs, there was also significantly lower resistance to the three classes of antibiotics (19.4%) compared to our results, where it was 41.3% for the three classes, 37% for the four, 4.8% five and one isolates (0.13%) in all six drug classes tested retrospectively (21). Only two isolated strains of *S. aureus* in our study showed complete sensitivity to all antibiotics tested.

CONCLUSIONS

Among the FHs in the Sarajevo Canton, a low percentage of *S. aureus* carriers are recorded. Our study confirmed that the potential risk of food poisoning caused by *S. aureus* same as secondary contamination of food by FHs in the Sarajevo Canton is low.

However, significant AMR of isolated strains indicates that Sarajevo Canton is following the global trend of rapid growth. Accordingly, it is necessary to limit the availability and uncontrolled use of antibiotics, the same as prescribing the therapy without the direct results of antimicrobial testing.

Based on the conducted research, we could not detect Staphylococcal enterotoxins and the origin of isolated strains and the nature/mechanisms of *S. aureus* resistance. Accordingly, the successful introduction of molecular methods into routine work would be crucial in tracing resistance mechanisms, monitoring of the health status of FHs, and investigating epidemiological relatedness during outbreaks.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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