Prevalence and biofilm forming potency of multi-drug resistant *Staphylococcus aureus* among food handlers in Arba Minch University, South Ethiopia

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Summary

Food borne diseases create pervasive health problems across the globe. The problem is severe in underdeveloped countries due to the difficulties in securing optimal hygienic food handling practices. Hence, this study is intended to explicate the prevalence, antibiotic resistance and biofilm forming profile of *Staphylococcus aureus* among food handler's working in Arba Minch University, South Ethiopia. A cross sectional study was conducted from April to June, 2015. Pre-tested struc-

tured questionnaire was used to collect data about socio demographic characteristics and other associated factors. The nasal samples were collected and examined for bacterial identification based on standard procedures. Bacterial isolates with highest and broadest rank of antimicrobial resistance and biofilm forming potency was chosen for partial 16s rRNA gene sequencing. All the laboratory experiments were performed in triplicates. A total of 281 food handlers were enrolled in the study with a response rate of 100%. Majority of the food handlers were females (72.6%) and were educated up to primary level (36.4%). Twenty (7.1 %) of them were found to be positive for nasal colonization of *S. aureus*, of which one strain (5.2%) was resistant to oxacillin. All the isolates showed high frequency of resistance to penicillin and cotrimoxazole. Results of biofilm forming assay revealed that out of 15 multidrug resistant isolates, two of them are potent biofilm producers. Further, DNA sequencing and BLAST analysis of the amplified ribosomal genes of two potent biofilm producers demonstrated a 98-99% homology with *S. aureus* 16S rRNA genes. In conclusion, food handlers are usually the main source of food contamination with *S. aureus*. Therefore, constant epidemiological surveillance through biannual routine tests and improvement of personal hygiene are recommended to control the transmission route.

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Key words
Food handlers, Staphylococcus aureus, nasal carriage, biofilm, multi-drug resistance

Introduction

Food-borne ailments cause morbidity, mortality and can lead to economic, social and political instability.[1] According to the World Health Organization (WHO), every year 1 in 10 people get infected from consuming tainted food and 420,000 die as a consequence.[2] The problem is more severe in developing countries due to difficulties in securing optimal hygienic food handling practices. It is estimated that in developing countries 70% of cases of diarrhea are associated with the consumption of contaminated food.[3] One of the possible explanations for an occurrence of food-borne ailments is incrimination of the food by an infected food handler. Food handlers or food employees are anyone who works in the cafeterias or restaurants and handling unpackaged food, food equipment or utensils, or food-contact surfaces.[4] Food handlers with poor personal hygiene could be a potential source of infections for many intestinal helminthes, protozoa, and pathogenic bacteria.[5,6] Indeed, handling food with bare hands can serve as a major factor for pathogen transfer to foods[7] and its estimated that 10 to 20% of food-borne disease outbreaks are due to contamination by the food handlers.[8]

According to International Commission on Microbiological Specifications for Foods, *Staphylococcus aureus (S. aureus)* is a risk group III food borne pathogen.[9] Earlier report envisaged that *Staphylococcal* food poisoning is considered as the third most prevalent cause of food borne illness worldwide.[10] Nasal carriage of *S. aureus* in food handlers is regarded as the major risk for *Staphylococcal* food poisoning.[11] Earlier studies corroborated that nasal *S. aureus* carried by food handlers become the source of contamination[11,12] and *S. aureus* colonized in the nasal region will automatically imply

its presence on the skin. It's also cited that *S. aureus* can survive on the skin and may be transmitted to cooked moist protein-rich foods, and become intoxication agents, if these foods are kept for several hours without refrigeration or stored in containers.[13,14] In addition, *S. aureus* are often resistant to several antibiotics. Thence, it is pivotal to investigate the nasal carriage rate of *S. aureus* among food handlers in order to prevent food contamination.[15] Survey of literature indicated that there is a scarcity in the reports pertaining to the food hygiene practices and nasal carriage rate of *S. aureus* in the study area. In this background, the present study is intended to assess prevalence, antibiotic resistance and biofilm forming potency of *S. aureus* among food handlers in Arba Minch University.

Methods

Study design, area and period

A cross sectional study was conducted at Arba Minch University which is located 505 kilometers to South of Addis Ababa. The study was carried out on food handlers participating in food preparation, dispatching and storage sections in Arba Minch University students' cafeteria from April- June, 2015.

Sample size determination and sampling technique

Sample size was calculated using sample size determination formula for the estimation of single population proportion. The P-value 0.21 was chosen from the previous study.[16] After considering 95% of confidence interval (z=1.96) and 5% marginal error (d=0.05); the initial sample size was 255 and, finally by computing a 10% (~26 subjects) non response rate, the final sample size was consolidated to be 281. In order to select representative groups, a proportional sample size was determined for each campus, and food handlers were selected randomly by lottery method from the roster lists of food handlers.

Data collection and laboratory processing

Data pertained to socio-demographic characteristics and personal hygiene practices of food handlers were collected by face to face interview method using pre tested structured questionnaire. The nasal swabs were aseptically collected using applicator stick tipped with cotton and wetted with normal saline (0.85% NaCl). The samples were collected by inserting the swab 2 cm into nasal vestibule and circulating six times against the anterior nasal mucosa. Afterwards, swabs were placed in sterile sleeve and transported to the Medical Microbiology and Parasitology Laboratory, Department of Medical Laboratory Science, Arba Minch University, on the same day.

Culture and biochemical identification

The nasal swabs collected from each food handlers were directly inoculated onto mannitol salt agar and blood agar (Oxoid, Basingstoke, Hamsphire, England). The inoculated plates were incubated face up for 24 hours at 35–37°C. Biochemical, morphological and physiological characteristics of isolated bacteria were ascertained by adopting standard laboratory methods including Gram staining, colonial morphology on different media, growth on selective media, catalase and coagulase tests.

Antibiotic susceptibility test

Antibiotic susceptibility profile was determined by Kirby-Bauer disk diffusion technique according to criteria set by Clinical Laboratory Standard Institute (CLSI), 2015 using Oxoid antibiotic discs. Briefly, inoculums were prepared by picking parts of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 barium sulphate solution. The test organisms were uniformly seeded over the Mueller-Hinton agar (Oxoid, Basingstoke, Hamsphire, England) surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium, and then incubated face up at 37°C for 24 to 48 hours. Diameters of the zone of inhibition around the discs was measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI, 2015. All intermediate readings were taken as resistant during data entry. The antibiotic disks including nitrofurantoin (30μg), penicillin (10IU), doxycycline (30μg), chloramphenicol (30μg), vancomycin (30μg), oxacillin (1μg), ciprofloxacin (30μg), erythromycin (15μg), gentamicin (10μg), cotrimoxazole (25μg) and amikacin (10μg) were used for the determination of antibiogram.

Biofilm forming assay

Qualitative and quantitative assay of biofilm forming potentials of all multi-drug resistance (MDR) isolates were performed as per the methodology described elsewhere.[17,18] Briefly, the MDR isolates (final concentration of 1×10^6 CFU) were cultured overnight at 37°C in Trypticase soy broth (TSB) supplemented with 1% glucose using 2 ml culture tubes.Tubes contained only 1% glucose supplemented TSB broth were used as control. Positive ALI-biofilm producers were identified visually

after overnight incubation based on the appearance of dense matt/slime formation on the surface of liquid media (Air - Liquid interface). After ALI inspection, the contents of each tube were vigilantly decanted and rinsed thrice with sterile physiological saline and air-dried in an inverted position for 1 hour at room temperature before being stained. To access the biofilm formation, 0.1% crystal violet solution (w/v) was added to the test and control dried tubes and kept for 10 min at room temperature. Further, the unstained dye was removed by washing thrice with sterile physiological saline and left for drying. A positive result was considered as the visible presence of crystal violet (CV) stained biofilm matrix accreted to the inner wall of the culture tubes by direct observation. Positive results were recorded after comparing with the negative control (un-inoculated culture tubes). For the quantification of accreted biofilm matrix, the same experiment was replicated in sterile polystyrene 96 well flat bottom micro-titre plates using the same isolates. Each well was seeded with 200 µL of fresh overnight culture of MDR isolates. The plate was covered and incubated at 37°C for 24 hrs. After incubation, wells were emptied by micropipette aspiration and washed thrice with sterile physiological saline in order to remove freely floating microorganisms. The accreted biofilm forming isolates were subsequently stained with 0.1% CV.Excessive stain was removed by washing thrice with sterile physiological saline and left for drying. Further, the biofilm formations were quantified by solubilization of the wells containing crystal violet stain in 200 µL of 95% ethanol. Afterwards, the absorbance (570 nm) of each well was measured using a Micro-plate reader (MR 9602). The un-inoculated wells containing broth alone were considered as blank control. In this study, MDR isolates with Optical Density (OD) 570 values greater than that of the negative control were considered positive for biofilm formation. According to the degree of biofilm formation, isolates were ranked in to three groups: non-adherent, optical density ≤ 0.111; moderately adherent, optical density ≥ 0.111 or equal to or <0.222; strongly adherent, optical density >0.222. The entire tests were carried out in triplicates and the data are expressed as mean ± standard deviation (SD) in order to validate the findings statistically.

Molecular identification of selected MDR *S. aureus*

Two bacterial isolates (056 & 126) with highest and broadest rank of antimicrobial resistance and biofilm forming potency was chosen for partial sequencing and phylogenetic analysis. Molecular identification of the potential candidate MDR isolates was based on partial 16S ribosomal RNA gene sequencing. Two selected MDR bacterial isolates were cultured in TSB at 37°C, and genomic DNA was extracted by the method described elsewhere.[19] The universal eubacterial 16S rRNA gene primers (27F: 5'AGAGTTTGATCCTGGCTCAG-3' 1492R: 5'-GGTTACCTTGTTACGACTT-3') were utilized for the PCR amplification of bacterial 16S ribosomal RNA gene. The PCR reaction mixture (25µl) consisted of the following reactants: 2.5µl of 10X Taq buffer containing 15 mM $MgCl_2$, 1µl $MgCl_2$ (25 mM),0.5 µl dNTP (10mM), 50 ng of genomic DNA (5 µl) and 0.125µl of Taq DNA polymerase (5U/µl), 1.25µl each of forward and reverse primers. Program used for amplification is initial denaturation at 95°C for 1 min (one cycle), followed by 35 cycles of denaturation at 95°C for 30 sec, primer annealing at 57.6°C for 1 min and extension at72°C for 1 min; final extension at 72°C for 5 min, and were stored at 4°C. The amplified products were resolved in 1% agarose gel, stained with ethidium bromide and the data was documented in the Gel doc instrument.

The amplified 16S ribosomal RNA gene product was purified using Gel extraction kit (Qiagen) as per the manufactures instruction. The purified PCR product was sequenced by Sanger's sequencing method.[20] The obtained 16S ribosomal RNA gene sequence from the two isolates were compared with other bacterial sequences in the NCBI (National Centre for Biotechnology Information) database by using BLAST (Basic Local Alignment Search Tool) to analyze pair wise homology. The sequences obtained were deposited in GenBank with accession numbers (KX523675 and KX523676). The sequences of bacteria showing similarity of about 90% or more were identified as the organism under study. The phylogenetic tree was constructed using MEGA 7 software (Molecular Evolutionary Genetic Analysis). The sequencing services were provided by Eurofins Genomics India Pvt. Ltd.

Data quality

Data quality was ensured at various activities of the study by following prepared standard operating procedure (SOP). One week prior to the actual data collection period, pre-test was conducted on 5% food handlers working in the cafeteria of Arba Minch hospital. The questionnaire was translated to the local language then translated back to english to ensure the consistency of the questionnaires. Completed questionnaires were inspected and corrected on daily base. Culture media was prepared according to manufacturer's instruction and sterility was checked by incubating representative of the batch at 35–37°C overnight and observing bacterial growth. Those batches of the media that showed growth were discarded. Control strain *S. aureus* (ATCC-25923) was used as a quality control for culture and to ensure the potency of antibiotic disks. The entire tests were carried out in triplicates.

Data analysis

Data was edited, cleaned, entered and analyzed using statistical package for social science (SPSS) version 20. Descriptive analysis such as frequencies and mean was used. Initially the association between each exposure and the presence of nasal carriage was assessed using the chi-square test, and odds ratio was computed to measure the strength of the association. Univariate and bivariate analysis were conducted and crude and adjusted odds ratio with 95% CI were calculated for statistical significance tests. Variables with significant at $P < 0.3$ in a bivariate analysis were considered for multivariate analysis through multiple logistic regression model to look their relative effect on the outcome variable by controlling other possible confounding factors. P-value of < 0.05 at 95% confidence interval was considered to indicate statistical association.

Results

Isolation and biochemical characterisation of nasal *Staphylococcus* isolates

In the present study, totally 20 isolates were retrieved from the food handlers. Results of Gram staining indicated that isolates were Gram positive cocci. The morphological studies revealed that the colonies were yellow, round, smooth, opaque and convex with a diameter of 1 mm. The bacterial isolates were non-motile, catalase and coagulase positive. Since the biochemical characteristics were similar for all the isolates, they were regarded as same strains. Finally, based on the overall results, these isolates were tentatively identified as *S. aureus*.

Socio demographic data

Amongst the 281 healthy food handlers, the overall prevalence of nasal carriage of *S. aureus* was 20 (7.1%). The lowest rate of nasal carriage was 4.9% in study participants with primary school education when compared with other educational status. The lowest rate of nasal carriage among service years was observed in <1 years which was zero, though there were no significance association between socio demographic variables and carriage of *S. aureus* (Table 1).

Hygienic practice of food handlers

In this study, the highest rate of nasal carriage was 15 (6%) related to utilization of bare hands to transfer cooked food, while the lowest rate of carriage was seen

Table 1

Socio-demographic characteristics in relation to *S. aureus* isolated from food handlers at Arba Minch University students' cafeteria, Arba Minch, South Ethiopia, April- June 2015 (n=281)

Demographic characters	*S. aureus* negative No. (%)	*S. aureus* positive No. (%)	Crude OR (95% CI)	P-value
Sex				
Male	72 (93.5)	5 (6.5)	1.46 (0.57-3.77)	0.43
Female	194 (95.1)	10 (4.9)	1.00	
Age in years				
< 20	19 (90.5)	2 (9.5)	1.07 (0.23-4.91)	0.93
21-35	166 (93.3)	12 (6.7)	1.00	
36-50	76 (98.7)	1 (1.3)	0.275 (0.06-1.22)	0.09
> 50	5 (100)	0	0.00	0.99
Years of service				
< 1 year	19 (100)	0	0.00	0.99
1-5 years	113 (91.1)	11 (8.9)	1.00	
6-10 years	85 (96.5)	3 (3.5)	0.48(0.17-1.37)	0.17
>10 years	49 (98)	1 (2)	0.16(0.02-1.25)	0.08
Educational status				
Illiterate	26 (100)	0	0.00	0.99
Primary school	97 (95.1)	5 (4.9)	1.00	
Secondary school	70 (93.3)	5 (6.7)	1.38 (0.47-4.08)	0.56
Higher than secondary school	73 (93.6)	5 (6.4)	1.15 (0.37-3.53)	0.81

among food handlers working with long nail and wearing jewelry while preparing food (2%). Albeit the food hygiene practices did not have any statistical association, 13 (7.5%) of the food handlers worked without jewelry, 3 (3.5%) did not wear hair cup when preparing food and 13 (5%) prepared food when suffering from diseases such as cough, cold, diarrhea and skin ulcer. While, 14 (6%) of the food handlers ne'er work with long nail (Table 2).

Hand washing habit of food handlers

Table 3 indicates the practices of hand washing with soap and water in relation to nasal carriage of the bacteria in different occasions. Results revealed that nine (7%) of the carrier food handlers washed their hands after toileting with soap and water. A great number of them reported to wash hands only with water after blowing nose 210 (94.2%).

Antimicrobial susceptibility profile of bacterial isolates

The antibiotic sensitivity profile of all isolates was confirmed using 11 antibiotics that are routinely prescribed in the study area. In the present study, the isolates varied in their susceptibility to all the antimicrobials tested. Percentage of isolates that are resistant to penicillin was 100%, followed by 40% in the case of cotrimoxazole. Invariably, all the isolates were 100%

Table 2 Hygienic practice of food handlers in relation of nasal carriage of *S. aureus* at Arba Minch University students' cafeteria, Arba Minch, South Ethiopia, April- June 2015 (n=281)

Hygiene practice	*S. aureus* negative No. (%)	*S. aureus* positive No. (%)	Crude OR (95% CI)	P-value
Wear jewelry when preparing food				
No	161 (92.5)	13 (7.5)	1.00	
Yes	105 (98.1)	2 (1.9)	5.83 (1.33-25.53)	0.02
Wear hair cup when preparing food				
No	84 (96.5)	3 (3.5)	0.55 (0.18-1.67)	0.28
Yes	182 (94)	12 (6)	1.00	
Wear gown when preparing food				
No	67 (97)	2 (3)	0.52 (0.15-1.82)	0.31
Yes	199 (94)	13 (6)	1.00	
Using bare hands to transfer cooked food				
No	24 (100)	0	1.00	
Yes	242 (94)	15 (6)	0.00	0.99
Touching food with cuts / wounds in hands				
No	85 (94)	5 (6)	1.00	
Yes	181 (95)	10 (5)	1.14 (0.44-2.94)	0.78
Preparing food when suffering from diseases				
No	27 (93)	2 (7)	1.00	
Yes	239 (95)	13 (5)	1.57 (0.438-5.61)	0.49
Certified in food training				
No	170 (94.4)	10 (4.6)	1.05 (0.41-2.71)	0.91
Yes	96 (95)	5 (5)	1.00	
Medical check up				
No	266 (95)	15 (6)	0.06	0
Yes	0	0	1.00	
Work with long nail				
No	203 (94)	14 (6)	1.00	
Yes	63 (98)	1 (2)	2.78 (0.63-12.23)	0.18

Table 3 Hand washing practices of food handlers in relation to nasal carriage of *S. aureus* at Arba Minch University Students' Cafeteria, Arba Minch, South Ethiopia, April- June 2015 (n=281)

Hand washing practices	*S. aureus* negative No. (%)	*S. aureus* positive No. (%)	Crude OR (95% CI)	P-value
After going to toilet				
No	0	0	0.00	0.99
Only with water	147 (97)	5 (3)	0.54 (0.22-1.36)	0.19
With water and soap	119 (92)	10 (8)	1.00	
Before food handling				
No	2 (67)	1(23)	5.06 (0.49-52.34)	0.17
Only with water	128 (96)	5 (4)	0.62 (0.24-1.61)	0.33
With water and soap	136 (94)	9 (6)	1.00	
In-between handling raw & cooked food				
No	10 (100)	0	1.18 (0.11-12.31)	0.89
Only with water	222 (94)	12 (6)	0.83 (0.23-2.95)	0.77
With water and soap	34 (92)	3 (8)	1.00	
After blowing nose				
No	36 (97)	1 (3)	1.13 (0.09-12.99)	0.93
Only with water	210 (94.2)	13 (5.8)	1.63 (0.21-12.75)	0.64
With water and soap	20 (95)	1 (5)	1.00	
After touching body parts				
No	46 (96)	2 (4)	0.51 (0.12-2.23)	0.38
Only with water	180 (95)	9 (5)	0.53 (0.18-1.56)	0.25
With water and soap	40 (91)	4 (9)	1.00	
After touching dirty materials				
No	0	0	0.00	0.99
Only with water	188 (96)	7 (4)	0.42 (0.17-1.04)	0.06
With water and soap	78 (91)	8 (9)	1.00	

susceptible to vancomycin, chloramphenicol and nitrofurantoin. The notable result is that among the penicillin resistant isolates 5% (n=1) were methicillin resistant *S. aureus* (MRSA) (Table 4). In the present study, 75 % (n=15) of the isolates were considered as MDR. Therefore, these strains were further chosen for elucidating the biofilm forming potential.

Biofilm forming potential of MDR

In the present study, out of 15 MDR isolates, 12 were found to be positive for biofilm, which formed dense aggregation around the tube wall. The biofilm formation rate of qualitative tube assay was estimated as 80%. In addition, among the 15 MDR isolate, 4 isolates formed a whitish matt at Air-Liquid Interface and also strongly adhered to the top liquid interface of the tubes (Table 5). The bacterial biofilm were further subjected to quantitative analysis at OD 570 nm. It was found that out of 15 MDR isolates, four of the isolates are strong biofilm producers, eight of the isolates are moderate biofilm producers and rest of the isolates failed to produce biofilm. Of the four strong biofilm forming isolates, two of them displayed high rank of biofilm production, registering an OD of 0.3497 and 0.3300 nm respectively (Figure 1). Based upon the results of biofilm formation using tube assay and microtiter assay, these two strongly biofilm producers were further taken-up for the molecular characterization using 16s rRNA gene sequencing.

Molecular identification of MDR isolates based on 16S rRNA gene sequencing

The 16S rRNA sequence of the MDR isolates (056 & 126) were blasted using megablast tool of GenBank (http://www.ncbi.nlm.nih.gov/). This revealed that the two MDR isolates were *S. aureus* strains. Representa-

Table 4

Antibiotic resistant pattern of *S. aureus* isolated from food handler's of Arba Minch University Students' Cafeteria, Arba Minch, South Ethiopia, April- June 2015

Antibiotics	Nasal isolates (n=20)	
	R/S	% of resistance
Vancomycin	0/20	0
Chloramphenicol	0/20	0
Nitrofurantoin	0/20	0
Ciprofloxacin	1/19	5.2%
Oxacillin	1/19	5.2%
Amikacin	2/18	11.1%
Gentamicin	2/18	11.1%
Erythromycin	5/15	25%
Doxycycline	6/14	30%
Cotrimoxazole	8/12	40%
Penicillin	20/0	100

n= total number of nasal isolates; R= Resistant; S= Sensitive

Table 5

Qualitative biofilm forming potentials of MDR *S. aureus* by ALI & Tube assay from the food handler's of Arba Minch University Students' Cafeteria, Arba Minch, South Ethiopia, April- June 2015

MDR Isolates	ALI Assay	Tube Assay
O56	+++	+++
126	+++	+++
1A	++	+++
4B	+	+++
082	–	++
192	–	++
141	–	++
146	–	++
1B	–	++
207	–	++
270	–	++
223	–	++

+++= Strong biofilm forming; ++ Moderate biofilm forming; + Weak biofilm forming; – No visible results

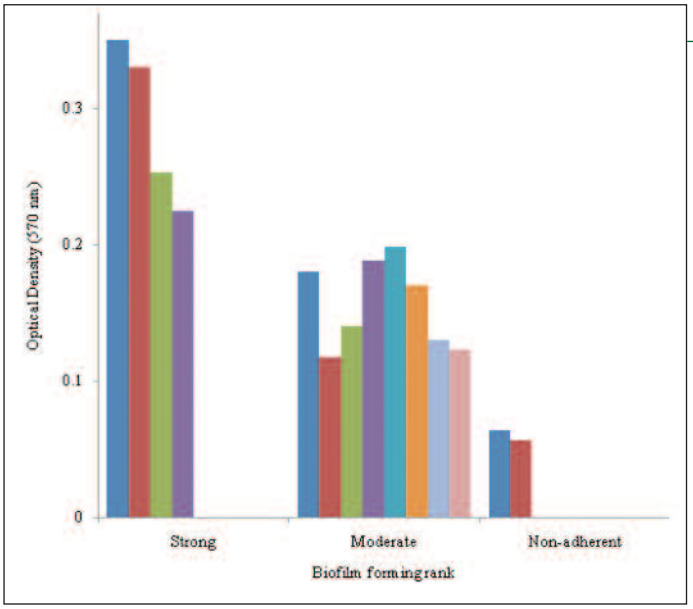

Figure 1

Quantitative biofilm forming potentials of MDR isolates by micro-titre plate assay isolated from food handler's of Arba Minch University Students' Cafeteria, Arba Minch, South Ethiopia, April-June 2015

tive of maximum homologous (98-99%) sequences of each isolate were obtained from seqmatch programme of RDPII and were used for the construction of phylogenetic affiliation (Figure 2 and 3). The 16S rRNA sequence of the MDR isolates was further analyzed using NCBI BLASTn tool with a query to limit the search closest relatives. Representatives of maximum homologous sequences from the search were used for the construction of phylogenetic tree using Maximum Likelihood method and unweighted pair group method with arithmetic mean (UPGMA) algorithm. The 16S rRNA gene partial sequence incurred in this study were submitted to the GenBank, EMBL in Europe and DNA Data Bank of Japan with assigned accession numbers KX523675 and KX523676.

Discussion

Poor personal hygienic practices of infected food handlers are most important contributor to the spread of food-borne diseases. Therefore, improvement of food worker's hand washing practices is critical for the reduction of food borne illness.[21] In this study 90% of the food handlers washed their hand frequently during food preparation which was much higher than results of 30.1% reported among the food handlers of Fayoum University.[22]

Contamination by food handlers is also probably a frequent occurrence in view of the high rate of human carriage. In this study, nasal swab culture of 281 food handlers had been investigated for the presence of *S. aureus* in their nostrils. It's posited that, nasal carriers of *S. aureus* probably originated from *Staphylococcal* infections.[23] The results of nasal culture revealed that *S. aureus* is carried by 20 (7.1%). The prevalence rate of our study was comparatively lower than the recent studies reported from Ethiopia and other part of the locales[16, 24-27] and higher than the prevalence rate done at elsewhere.[28] Nasal carriage rates reported by several researchers vary and the variation could be attributed to the ecological differences of the study population. In addition, isolation of *S. aureus* from the nose of food handlers in this study envisages possibilities for the transmission of this organism from nose to hand.

Albeit the *S. aureus* can inflict severe diseases in human, it may also be a member of the normal flora of the nasal cavity.[29] If the nasal colonized *S. aureus* is

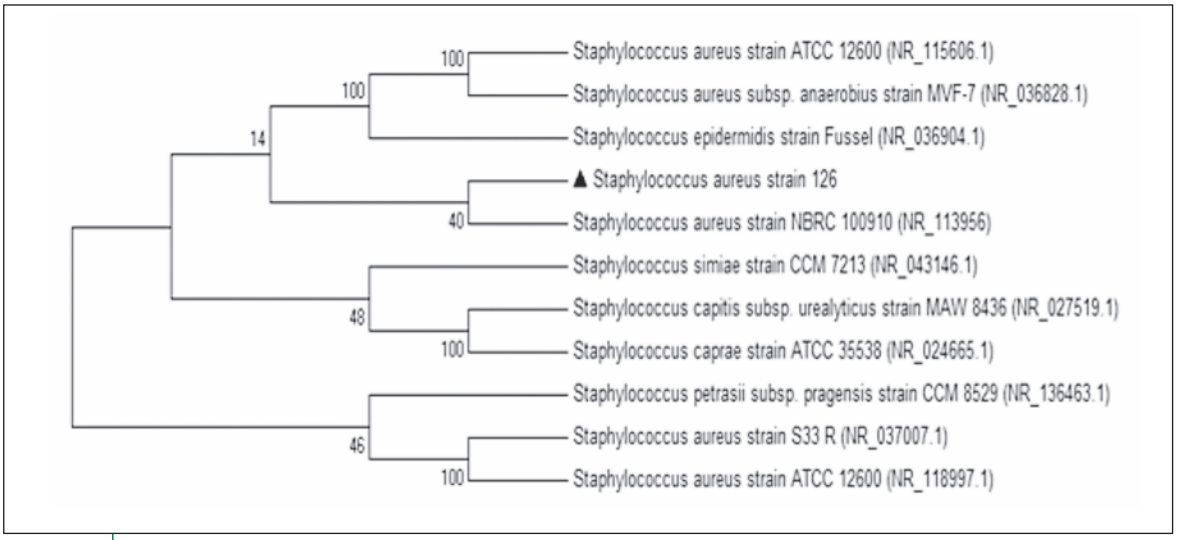

Figure 2 Evolutionary relationships of taxa of strain 126 isolated from food handler's of Arba Minch University Students' Cafeteria, Arba Minch, South Ethiopia, April- June 2015.

The evolutionary history was inferred using the UPGMA method.[34] The bootstrap consensus tree inferred from 50 replicates is taken to represent the evolutionary history of the taxa analyzed.[35] Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (50 replicates) are shown next to the branches.[35] The evolutionary distances were computed using the Maximum Composite Likelihood method[36] and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1405 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.[37]

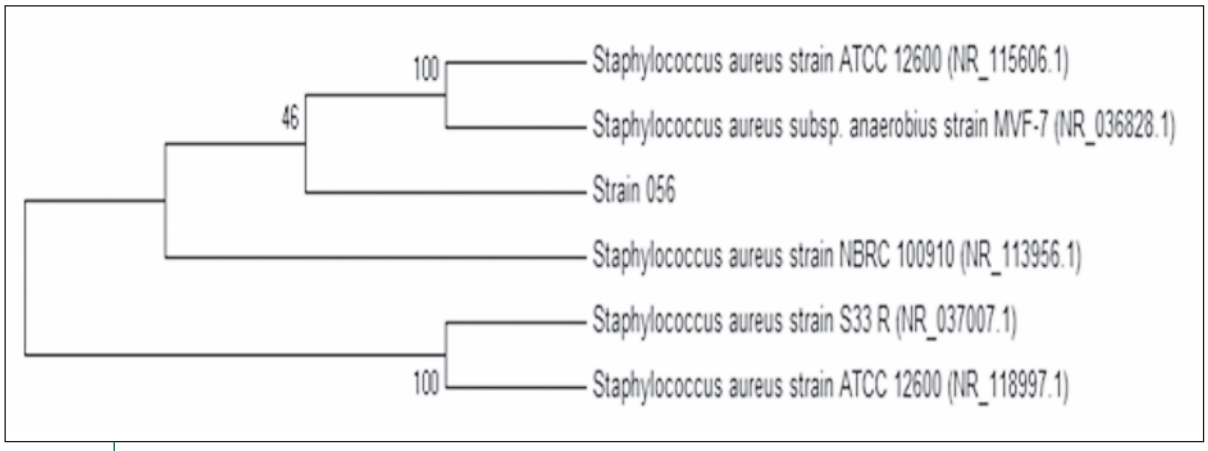

Figure 3 Evolutionary relationships of taxa of strain 056 isolated from food handler's of Arba Minch University Students' Cafeteria, Arba Minch, South Ethiopia, April- June 2015.

Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model.[38] The bootstrap consensus tree inferred from 50 replicates is taken to represent the evolutionary history of the taxa analyzed.[35] Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (50 replicates) are shown next to the branches.[35] Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+ 2nd+ 3rd+ Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1408 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.[37]

an enetrotoxin producing strain, it can incur chances of unfortunate consequences like food poisoning outbreaks in the students' community. Howbeit, in the present study enterotoxin production by isolated *S. aureus* were not investigated.

The emergence of antibiotic resistance amongst *S. aureus* plays a vital role in the epidemiology of *Staphylococcal* infections.[30] Totally, eleven routinely prescribed antimicrobial agents in the study area were selected to examine the resistance pattern of *S. aureus*. It was found that nasal isolates showed variable degree of resistance. In this study, invariably all the nasal isolates of *S. aureus* displayed resistant to penicillin (100%) whereas 95% (n=19) of isolates showed resistance against ampicilin and 40% (n=8) against cotrimoxazole which is higher than in a study done at Gondar, Northwest Ethiopia.[16] Of the total isolates, one strain (5.2%) of *S. aureus* was resistant to oxacillin. It is a well established fact that infections associated with MRSA have emerged over the past few decades causing challenges for both patients and medical practitioners due to the limited availability of safe therapeutic options. The rate of resistance in our study was lower than [4(9.8%)] a study done in Gondar, Northwest Ethiopia.[16] The possible reason for this differences may be due to the cross contamination of hospital acquired drug resistance strains in the study done at Gondar. Recent meta-analysis study on the prevalence of MRSA from diverse study subjects in Ethiopia were 32.5%.[26] In our study, invariably all isolates were sensitive to vancomycin, chloramphenicol and nitrofurantoin which is well nigh resembled with previous findings from Ethiopia.[16] Howbeit, in contrast to our results, a study conducted in Botswana evinced that 9 (27.3%) of the isolates were resistant to vancomycin.[27] The differences might be due to *S. aureus* strains from different anatomical sites on food handlers in Gaborone, Botswana.

Species of the genus *Staphylococcus* are well known producers of biofilm. It is envisaged that the biofilm forming *S. aureus* pose a risk for public health by generating resistance against antibiotics, disinfectants and immune-defense mechanism.[31] Therefore, inspection of biofilm formation could be a valuable indicator for the pathogenicity of *Staphylococci*. The results of the present study envisaged that 80% of the isolates had the ability to form biofilm and 33% of them are prominent biofilm producers. The results ob-

tained are in accordance with other studies.[32,33] Further, molecular characterization based on partial 16S rRNA gene sequence revealed that the potent biofilm forming MDR isolates were *S. aureus*.

Limitation of the study

As this is a cross sectional study we are unable to conclude whether *S. aureus* colonization is intermittent or persistent and enterotoxin was not identified because of resource limitation. In addition, the resistant patterns of oxaciilin resistant strains were not re-confirmed by cefoxitin test.

Conclusion

This is the first report on the prevalence, antimicrobial resistance pattern, biofilm forming potency and molecular characterization of the nasal associated *S. aureus* isolated from food handlers of Arba Minch province. The overall prevalence rate was 7.1%. Of the isolates, about 75% (15 strains) of them were considered as MDR and 5.2% are found to be MRSA. Invariably, all the isolates were susceptible to vancomycin, chloramphenicol and nitrofurantoin. Eighty percent of MDR isolates were biofilm producers. It's evidenced that in addition to the contaminations pertained to food handlers, equipments and environmental surfaces can also serve as a potential source of contamination. Therefore constant epidemiological surveillance through biannual routine tests and improvement of personal hygiene are recommended to control transmission route. Further research is being underway on the aspects of elucidating enetrotoxin producing ability of MDR *S. aureus*.

Abbreviations

ATCC, American Type Culture Collection; ALI, Air Liquid Interface; BLAST, Basic Local Alignment Search Tool; CI, Confidence Interval; CLSI, Clinical Laboratory Standard Institute; EMBL, European Molecular Biologist Laboratory, MEGA, Molecular Evolutionary Genetic Analysis; MRSA, Methicillin Resistance *S. aureus*; NCBI, National Centre for Biotechnology Information; PCR, Polymerization Chain Reaction; RDP, ribosomal database project; SOP, Standard Operating Procedures; SPSS, Statistical Package for Social Sciences; UPGMA, unweight pair groupmethod with arithmetic mean

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Availability of Data and Materials

The original data for this study is available from the corresponding author

Authors' contributions

MM: Primary researcher conceived the idea for this study. MM, GA, AM and MS: participated in data collection, conducted data analysis, drafted and finalized the manuscript for publication. AM and AI involved in molecular characterization of MDR isolates. All authors read and approved the final manuscript.

Competing interests

All authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study protocol was ethically approved by review boards of Arba Minch University, College of Medicine and Health Sciences with project code of GOV/AMU/TH.5-2/CMHS/MLS/02/07. Letter of cooperation was written to each leader of the cafeteria. Informed verbal consent was obtained from each study participant. Strict confidentiality was maintained during the interview process as well as anonymity was kept during data processing and report writing.

Περίληψη

Επιπολασμός και μελέτη δυνατότητας σχηματισμού βιομεμβράνης σε πολυανθεκτικά στελέχη *Staphylococcus aureus* που απομονώθηκαν από χειριστές τροφίμων στο Πανεπιστήμιο ArbaMinch, Νότια Αιθιοπία

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Οι τροφιμογενείς λοιμώξεις αποτελούν ένα σοβαρό πρόβλημα δημόσιας υγείας σε παγκόσμια κλίμακα, με εντονότερο το πρόβλημα στις υπό ανάπτυξη χώρες, λόγω δυσκολιών στην εξασφάλιση βέλτιστων πρακτικών υγιεινής διατροφής. Η παρούσα μελέτη αφορά την επικράτηση, την αντοχή στα αντιβιοτικά και τη δυνατότητα σχηματισμού βιομεμβράνης στελεχών *Staphylococcus aureus* που απομονώθηκαν σε χειριστές τροφίμων που εργάζονται στο Πανεπιστήμιο Arba Minch της Νότιας Αιθιοπίας. Η συγκεκριμένη μελέτη πραγματοποιήθηκε από τον Απρίλιο έως τον Ιούνιο του 2015. Χρησιμοποιήθηκε ένα προκαταρκτικά δομημένο ερωτηματολόγιο για τη συλλογή δεδομένων σχετικά με τα κοινωνικο-δημογραφικά χαρακτηριστικά των χειριστών. Στη συνέχεια συλλέχθηκαν ρινικά επιχρίσματα και εξετάστηκαν σύμφωνα με τις κλασικές μικροβιολογικές μεθόδους για *S. aureus*. Τα βακτηριακά στελέχη *S. aureus* που απομονώθηκαν ελέγχθηκαν για την αντοχή τους στα αντιβιοτικά με τη μέθοδο διάχυσης δίσκων αντιβιοτικών σε άγαρ και μελετήθηκε η δυνατότητα παραγωγής βιομεμβράνης. Τα ανθεκτικά στελέχη με θετική τη δοκιμασία παραγωγής βιομεμβράνης επιλέχθηκαν για φυλογενετική ανάλυση με αλληλούχηση του 16s rRNA γονιδίου. Όλα τα εργαστηριακά πειράματα εκτελέστηκαν εις τριπλούν. Συνολικά 281 χειριστές τροφίμων συμμετείχαν στη μελέτη με ποσοστό απόκρισης 100%. Η πλειονότητα των χειριστών τροφίμων ήταν γυναίκες (72,6%) πρωτοβάθμιας εκπαίδευσης (36,4%). Είκοσι (7,1%) βρέθηκαν θετικές για ρινικό αποικισμό από *S. aureus*, εκ των οποίων 1 (5,2%) ήταν αποικισμένη με στελέχη ανθεκτικά στην οξακιλλίνη. Όλα τα στελέχη έδειξαν υψηλό ποσοστό αντοχής στην πενικιλλίνη και κοτριμοξαζόλη. Τα αποτελέσματα της ανάλυσης σχηματισμού βιομεμβράνης έδειξαν ότι από τα 15 πολυανθεκτικά στελέχη, δύο ήταν ισχυροί παραγωγοί. Περαιτέρω ανάλυση του 16s rRNA γονιδίουτων δύο ισχυρών παραγωγών βιομεμβράνης έδειξε 98-99% ομολογία. Συμπερασματικά, οι χειριστές τροφίμων είναι συνήθως η κύρια πηγή μόλυνσης των τροφίμων, ενώ ο εξοπλισμός και οι περιβαλλοντικές επιφάνειες μπορεί επίσης να είναι πηγές μόλυνσης με *S. aureus*. Ως εκ τούτου, συνιστάται η συνεχής επιδημιολογική παρακολούθηση μέσω εξαμηνιαίων δοκιμών ρουτίνας και η βελτίωση της προσωπικής υγιεινής για τον έλεγχο της οδού μετάδοσης.

Λέξεις κλειδιά

χειριστές τροφίμων, Staphylococcus aureus, ρινική φορεία, βιομεμβράνη, ανθεκτικά στελέχη

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