Bacterial Assessment and Their Antibiotic Sensitivity Patterns of Nasal and Hand Swab Specimens From Food Handlers in Hospital Compounds in Mandalay, Myanmar: A Cross-sectional Descriptive or Observational Study

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Research

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Abstract

Background: Microbial contamination of food can occur from contaminated or infected food handlers.

Methods: This study aimed to assess type of bacteria and their antibiotic sensitivity pattern of nasal and hand swab samples from food handlers working in hospital compounds, Mandalay city, Myanmar. All eligible food handlers working in the government hospital compound, Mandalay City, Myanmar, were subjected to a cross-sectional study conducted from May to August 2018. Hand and nasal swabs were collected from of food handlers for bacterial isolation and identification. Antibiotic sensitivity patterns of isolated bacteria were determined.

Results: Among 111 participants, 92 (82.9%) were bacteria-positive by hand swabs and 77(69.37%) were positive by nasal swab. Among *Staphylococcus aureus* isolated from nasal swabs, just over a half (51.2%) were resistant to oxacillin (probably methicillin resistant *Staphylococcus aureus*). There were association between hand contamination and nasal bacterial growth ($p<0.001$).

Conclusion: Bacterial contamination status of the food handler working in hospital compound, Mandalay city, Myanmar, was extremely high.

Background

Food become unsafe when it was contaminated with harmful organisms and/or chemical ingredients. Unsafe food generates a vicious cycle causing food-borne diseases ranging from self-limiting or mild gastrointestinal symptoms (such as abdominal pain, mild diarrhoea) to severe form of diseases like severe diarrhoea, dysentery or cancers [1]. The chances of contamination of food mainly hang on food handlers' behaviours & hygiene practices [2]. Often unhealthy food handlers who have not received appropriate health inspection can be sources of food-borne diseases to their consumers [3–5].

The hospital environment engaged by diseased patients generally can turn out to be contaminated [6]. People with extreme age (older and children or infants) and special conditions such as cancer, diabetes, immunocompromised and transplanted patients are added liable to involvement food-borne diseases than the others [7]. Hospital eating establishments deliver to many residents' groups covering of medics, patients, nurses, hospital staffs, health pupils, guests of patients etc. There is a additional risk of food-borne illness occurrences if basic healthy and hygienic practices are not preserved [8]. American Centers for Disease Control and Prevention (CDC) estimated 48 million people suffer from a foodborne infection, 128,000 persons are hospitalized, and 3,000 people die every year in the United States [9]. It was stated that > 4,000 food-borne outbreaks including water-borne outbreaks in Europe 2016 [10]. In Myanmar, diarrhea and dysentery are ordered fourth national priority diseases. The expansion of food-borne illness has been inflamed, commonly linked with outbreaks or epidemics, and threatens international public health safety and concern [11].

In Ethiopia, *Staphylococcus aureus* (*S. aureus*) and other bacteria such as *Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Pseudomonas aeruginosa* were the common bacterial contaminants among food handlers [12]. Food handlers can transmit infection directly from their hands to eye, nose, mouth and skin or indirectly transmitting microbes via handling of food or water. In addition, hands cleanliness is one of the most significant and efficient points to achieve safe food handling to avoid contamination with dangerous microorganisms. In this research, we determined bacterial types and their antibiotic sensitivity pattern isolated from nose and hands of food handlers working in the government hospital compounds, Mandalay City, Myanmar. The data generated will deliver us important evidence and information for development and determination of further approaches or projects to be taken to progress healthiness of food handlers proposing to improve food safety in the government hospitals in Myanmar.

Methods

Study Area This study was conducted in 17 eating establishments in 8 government hospitals at Mandalay City. Myanmar.

Study Population The study population comprised of all eligible Myanmar food handlers who involved in food process of preparation and cooking and are working in the eating establishments in government hospital compounds, Mandalay city.

Sample Size Estimation Sample size was determined by means of $n = \frac{Np(1-p)z^2}{d^2(N-1)+p(1-p)z^2}$ for finite population proportion. Population size (N) was assumed as 150 Myanmar food handlers working in the government hospital compounds, Mandalay city, Myanmar. The expected proportion of satisfactory practice (p) of 54.1% was based on the data among 172 food handlers of selected restaurants in Nay-pyi-taw with z 1.96 (95% confidence interval) and precision (d) 5%. Therefore, the calculated minimum required sample size (n) is 108.

Study Design This was a cross-sectional descriptive or observational study designed to determine type of bacteria and their antibiotic sensitivity patterns of nasal and hand swab samples collected from food handlers working in the eating establishments in the government hospital compounds, Mandalay city, Myanmar

Selection Criteria Myanmar food handlers working in hospital compounds for preparation and cooking of food were selected.

Data Collection Method and Tools

Firstly, the investigator explained pros and cons of this study and obtained written informed consents from the participants. The investigators cleaned and washed his hands with soap and water and wore gloves before sample collection using hand and nasal swabs to prevent cross contamination. Hand swab samples were taken from the participants by rolling several times over the inter-digital spaces, dorsal and ventral aspect of hands. In brief, a sterile cotton wool swab soaked with sterilised normal saline were applied to the indicated sites after the participant washed their hands with water and dried in the air. Nasal swab samples were taken from both anterior nares by touching 2 cm inside from the nasal orifice and softly rotate in contradiction of the anterior nasal mucosa for three to five times according to the guideline of “Specimen Collection Procedures Manual For National Health And Nutrition Examination Survey, 2000” [13]. The swab stick was put in Stuart’s transport media and location, age, sex, date and sample numbers are labelled. After that collected samples were sent to the Department of Microbiology Laboratory, University of Medicine, Mandalay (UMM) as soon as possible for bacterial isolation and identification. These procedures were completed with the support of microbiologist from this Microbiology department.

The swab samples were inoculated onto Blood, MacConkey and Nutrient agar plate. All the plates were incubated at 37°C for overnight. After inoculation onto culture plates, the specimens were smeared on clean glass slides, heat fixed, stained with Gram’s stain and examined under an ordinary light microscope fitted with x100 oil immersion lens to examine the presence of inflammatory cells and bacteria. If gram-positive cocci arranged in grape like clusters were present, the colony was sub-cultured onto mannitol salt agar (MSA) plate supplemented with 5% v/v egg yolk and incubated at 37ºC for 24 hours.

Antibiotic susceptibility test was performed against all *S. aureus* colonies according to the modified Kirby-Bauer disc diffusion method. The results were interpreted as sensitive, intermediate and resistant using a zone size interpretation chart at 24 hours following CLSI criteria [guidelines of the Performance Standards for Antimicrobial Disk Susceptibility Tests from (CLSI, 2011)].

Five common clinically used antibiotic (amikacin, levofloxacin, ceftriaxone, clindamycin and amoxicillin-clavulanic acid) discs were placed in equal spacing with a sterile needle tip. Other three antibiotic (cefoperasone-sulbactam, vancomycin and oxacillin) discs were tested randomly. Each disc was pressed down to ensure complete contact with the agar surface. Then the plate was inverted and placed in an incubator set at 37ºC. After overnight incubation, the diameter of each inhibitory zone was measured in millimetre using a ruler. The size of inhibitory zone was interpreted according to the chart of inhibitory zone sizes of the Clinical and Laboratory Standards Institute (CLSI, 2011).

Data Processing and Analysis Data was checked daily after collection for completeness and correction. Data entry was done by using the software EpiData 3.1. Statistical analysis was performed with statistical software StataSE 16. Mean and standard deviation were used to summarize the normally distributed continuous variables, whereas Median and interquartile range were used for non-normally distributed continuous variables. The normality of the variables was checked by skewness/kurtosis test and histogram. Categorized continuous variables and categorical variables were summarized by using frequency distribution tables. Chi-squared test (χ^2) was used for categorical variables. Fisher’s exact test was used for categorical variables when the expected value was less than 5. The significance level was considered as 0.05.

Results

For hand contamination of the food handlers, 111 samples of hand swabs were taken for bacteriological examination. The results show that 92 (82.88%) samples showed the presence of hand contamination. Concerning hand swabs of the food handlers (Table 1), the major contaminant was *S. aureus* (42.35%) of total hand swab samples.

Table 1
Bacteria isolated from hand swabs

Bacteria isolated from hand swabs	Frequency	Percent
Staphylococcus species	53	47.76
- *S. aureus*	47	42.35
- Coagulase negative staphylococci	6	5.41
Escherichia coli	9	8.11
Klebsiella spp.	6	5.41
Acinetobacter spp.	3	2.70
Pseudomonas spp.	1	0.90
Mixed organisms	10	9.00
Others	10	9.00
Total	92	82.88

For nasal bacterial growth, 111 samples of nasal swabs were taken for bacteriological examination. It revealed that 77 (69.37%) of the samples showed nasal bacterial growth. Concerning nasal swabs of the food handlers (Table 2), majority (54.95%) of total hand swab samples was *S. aureus*.

Table 2
Bacteria isolated from nasal swabs

Bacteria isolated from nasal swabs	Frequency	Percent
Staphylococcus species	67	54.95
S. aureus	61	5.41
Coagulase negative staphylococci	6	
Gram-positive bacilli	6	5.41
Klebsiella spp.	2	1.80
Acinetobacter spp.	2	1.80
Total	77	69.37

Antibiotics susceptibility of *S. aureus* (Fig. 1) isolates were tested against amikacin, levofloxacin, ceftriaxone, clindamycin, cefoperasone-sulbactam, vancomycin, amoxicillin-clavulanic acid and oxacillin. Almost all (96.72%) were sensitive to amikacin and levofloxacin, whereas sensitivity to ceftriaxone, clindamycin, cefoperasone-sulbactam and vancomycin were 91.8, 90.2, 88.9 and 85.2%, respectively. About a half (49.2%) of them were sensitive to amoxicillin-clavulanic acid, while one third (31.2%) of them were resistant to it. Just over a half (51.22%) of *S. aureus* isolates were oxacillin resistant *S. aureus* (ORSA), which are probably be methicillin-resistant *S. aureus* (MRSA).

Among 111 food handlers examined, 98 (88.3%) of the samples showed bacterial contamination of food handlers in hospital compounds. The majority 71(77.2%) of the food handlers who has hand contamination were nasal bacteria positive and 21(22.8%) were negative of nasal bacteria. An association between hand contamination and nasal contamination of the food handlers was statistically significant (X^2=15.41, $p < 0.001$) (shown in Table 3).

Table 3
Association between hand contamination and nasal contamination of the food handlers

Hand Contamination	Nasal Contamination		X^2	p value
	Absent n (%)	Present n (%)	15.41	< 0.001
Absent	13 (68.4)	6 (31.6)		
Present	21 (22.8)	71 (77.2)		

Discussion

According to this study, bacterial contamination was present in (88.29%) of hand and nasal swabs of the food handlers. In Serbia, (24.6%) and (21.6%) smear positive central and distributive kitchens from hands and work clothes, respectively [14]. The study in Brazil found that (24.4%) of coagulase-positive staphylococci and (75.6%) coagulase-negative staphylococci in analysing nasal and hand swabs of 82 food handlers [15]. Therefore, bacterial contamination in the present study was higher than those of other countries.

Hand contamination of the food handlers

Regarding *Staphylococcus* spp. contamination on hands, (47.8%) of the food handlers were contaminated with *Staphylococcus* spp. in which (42.4%) was *S. aureus* and (5.4%) was coagulase negative *Staphylococcus* species. The finding of the present study is consistent with the study where there was *Staphylococcus* spp. (56.9%) of the nail cultures, and another study where the presence of *S. aureus* on hands of the food handlers in schools expressed (53.3%) [3, 16]. In contrast, other studies who reported lower *S. aureus* prevalence are (30.1%) [17], (29.2%) [18], (23.5%) in Ethiopia [12], (17.5%) in Makkah, Saudi Arabia [19], (16.5%) from their finger-nail contents [20], (12.6%) isolated from hands of Iranian food handlers [21], (11.1%) [22] and (5.6%) [23]. This difference in contamination may be due to different populations and areas of the study.

In this study, the second most prevalent contaminant isolated from the hands of the studied group was *Escherichia coli* (8.11%). It is considered an enteric pathogen that are supposed to be capable of being spread by the food handlers in hospital compounds. The current finding has higher prevalence than the study (3.1% and 2.5% respectively) [19, 20]. This result which reflects hand contamination with faecal matter pointed to insufficient and poor handwashing habits among the food handlers.

Nasal bacterial growth of the food handlers

When nasal swab culture was examined, *S. aureus* was found in (54.95%) and coagulase negative staphylococcus was in (5.41%). The prevalence of nasal staphylococci carrier in this study is higher than that in the study [24] in Brazil (40.8%) and in Iraq (30.1%) [17]. In the study in Spain, 27.6% of 300 food handlers were found to be nasal coagulase positive staphylococci carriers [25]. The study in Kuwait city [26] revealed that (26.6%) of 500 restaurant workers were nasal *S. aureus* carriers. In Brazil, (24.4%) and (75.6%) of nasal and hand swabs of 82 food handlers were positive for coagulase-positive and coagulase-negative staphylococci [27]. There were (19.8%) and (10.4%) isolates from the nasal cavity of the food handlers respectively [22, 23]. In contrast, the result of the present study is higher than those studies.

The current results presented that (1.8%) of the nasal swabs of the food handlers were *Acinetobacter* spp. It is a common cause of nosocomial or hospital acquired infection in Serbia. It is becoming a problematic due to their quick progress of drug resistance and high fatality rates (20-60%). Its transmission is possible via person-to-person connection, water contamination, food borne and contaminated hospital and surgical equipment [28].

Pattern of antibiotic sensitivity of *Staphylococcus aureus* isolates from nasal carriers of the food handlers

The detection of antibiotics-resistant *S. aureus* in nasal specimen is a public health attention as they can serve as sources of drug resistant *S. aureus* transmission in the community. In the present study, (1.64%) were resistant to amikacin. The study in Brazil stated (4.0%) were resistant to amikacin and those were higher than this study [24]. There was no resistant strain for cefoperasone-sulbactam antibiotic in the current study. Amikacin and cefoperasone-sulbactam are antibiotics most often used for intending to treat severe respiratory infections or multidrug-resistant tuberculosis in Myanmar. The reason of few percentage of drug resistant is that there are no oral form and also needs doctor prescriptions.

In the current study, (1.64%) was resistant to levofloxacin and (6.56%) was resistant to ceftriaxone. These two drugs are also used as first line drugs in respiratory tract and gastro-intestinal tract infections according to hospital infection control guideline before getting specific antibiotics sensitivity results. In other study, (97.68%) isolates were resistant to ceftriaxone [29]. Therefore, those were so higher resistant percentage than the current study.

In the present study, (9.84%) and (18.7%) isolates were resistant to clindamycin and amoxicillin-clavulanic acid, respectively. Clindamycin and amoxicillin-clavulanic acid are antibiotics most often used for the treatment of respiratory tract infection in Myanmar. Most Myanmar people takes these two drugs to relieve signs and symptoms of upper respiratory tract infection (e.g. sneezes, cough, sputum production) without prescription of doctors. In the study of Udo in Kuwait city, only (2.0%) were resistant to clindamycin [30]. Other study stated (27.14%) of nasal swab samples were resistant to amoxicillin-clavulanic acid and those were higher than this study [31].

(5.56%) isolates in the present study was resistant to vancomycin but there were no resistant to vancomycin tested in food handlers working the community study [22]. In the study in Botswana, (6.0%) were resistant to vancomycin [32]. In the current study, the prevalence of nasal carriers of ORSA (probably MRSA) was found to be 21 (18.92%) of total 111 food handlers and (51.22%) of *S. aureus* were resistant to oxacillin. But *S. aureus* strains were resistant to oxacillin (22.4 %) [32] and (0%) [22]. The current study result was higher than the previous studies. This difference might be due to different microbiological method of confirming MRSA. In the current study, MRSA is identified only by oxacillin disc diffusion test and not confirmed by cefoxitin disc diffusion test, MRSA latex agglutination test and PCR. Foods may be contaminated by human strains of MRSA present in meat processors and other food handlers [33]. Due to regional variation, choice of antibiotics should be guided by local susceptibility patterns.

Association of bacterial contamination among the food handlers in hospital compounds

There was an association between hand contamination and nasal contamination ($p<0.001$). Similar to the present study, food handlers who carry *S. aureus* in their noses are a likely source of hand contamination in their workplace [34].

Conclusions

This study revealed high in bacterial contamination and antibacterial resistance to *S. aureus* from the food handlers, justifying the screening testing methods to detect carriers and protect people from staphylococcal food poisoning before appointing and during working as food handlers in hospital compound. Misuse of antibiotics pave the way for the *S. aureus* to cause infections and to develop drug resistance. Moreover, there was seen significant association between hand contamination and nasal bacterial growth, and high in bacterial contamination status of the food handler working in hospital compound, Mandalay city, Myanmar.

List Of Abbreviations

CDC American Centers for Disease Control and Prevention

CLSI Clinical and Laboratory Standards Institute

MRSA Methicillin-Resistant *Staphylococcus aureus*

ORSA Oxacillin Resistant *Staphylococcus aureus*

UMM University of Medicine, Mandalay

Declarations

Ethics approval and consent to participate Permission to conduct this study ID No. 166 (MPTM)/ UMM/ 2018 was gained from Research and Ethics Committee of the University of Medicine, Mandalay (UMM) Myanmar. All eligible food handlers were explained about the aims and objectives of the study, hand and nasal swabs sample taking procedures, possible risks and benefits of participation in this study and duration of the conduct time. They were asked to participate in the study voluntarily with their signed written informed consent.

Consent for publication Not applicable

Competing interests N/A

Availability of data and materials The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions STA conceived and designed the study. STA and AAN collected the primary data. STA carried out the statistical analyzes. All authors participated in interpretation of the results, and prepared the first draft of manuscript. STA and AAN reviewed and edited the manuscript. All authors critically reviewed, revised, and approved the final manuscript.

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Animal Studies N/A

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Figures

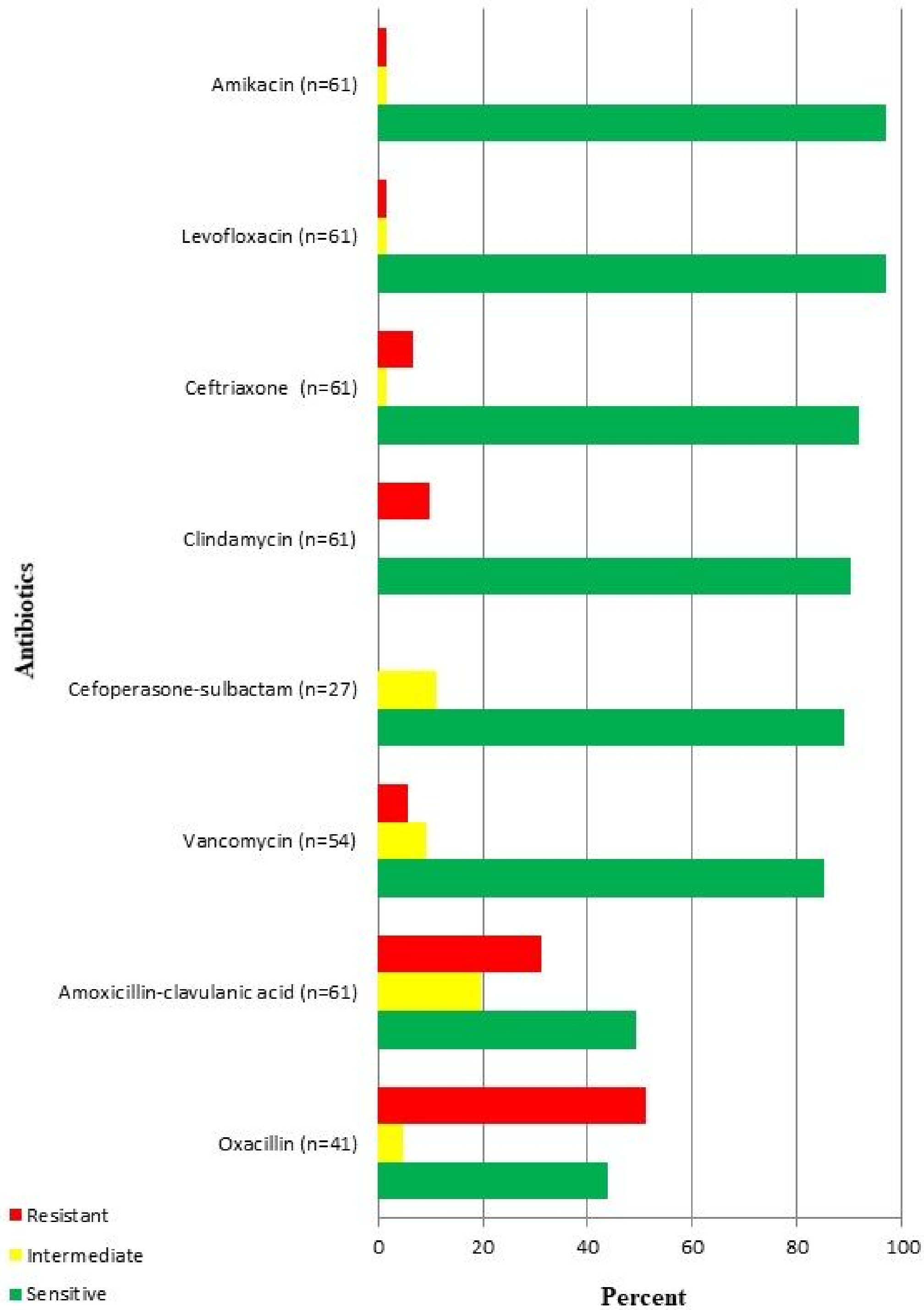

Figure 1

Pattern of antibiotic sensitivity of Staphylococcus aureus isolates from nasal carriers

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