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Antimicrobial resistance of *Escherichia coli* and *Staphylococcus aureus* isolated from food handler's hands

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ABSTRACT

The aim of this study was to determine the antimicrobial resistance of *Escherichia coli* and *Staphylococcus aureus* isolates from food handlers' hands at primary schools in Hulu Langat district, Selangor (Malaysia). Disc diffusion methods were used to examine the antimicrobial resistance of the bacteria by using ten types of antibiotic discs with different concentrations. The results show that the prevalence of *S. aureus* (65.88–74.12%) was far higher than the prevalence of *E. coli* (9.41–14.12%). The percentage isolates of *E. coli* that were resistant to the antibiotics was 85.71% Penicillin and Chloramphenicol, 57.14% Sulfamethoxazole-Trimethoprim, Ampicillin and Trimethoprim, 28.57% Kanamycin and Tetracycline and 14.29% Ciprofloxacin. All of the isolates had shown susceptible to Gentamicin and Nitrofurantoin. For *S. aureus*, the percentage isolates that were resistant to the antibiotics was 72.30% Ampicillin, 53.38% Penicillin, 4.73% Nitrofurantoin,1.35% Chloramphenicol and Trimethoprim and 0.68% Kanamycin and Tetracycline. None of the isolates had shown resistant to Ciprofloxacin, Sulfamethoxazole-Trimethoprim and Gentamicin. Multidrug resistant *Escherichia coli* represented a high percentage (85.71%) of the total positive strains revived whereas multidrug resistant *S. aureus* strains were only 5.41% of the total positive strains. The existence of multidrug resistant bacteria is quite worrying as they may pose serious threat to the patients. Hence, the microbiological quality of food handlers' hands from foodservice operations should be maintained in a good condition to reduce the existence of multidrug resistance bacteria.

1. Introduction

Antimicrobial resistant bacteria are a serious issue as they could pose a serious threat to the patients. Antibiotics are antimicrobial agents that are produced naturally by microorganisms to inhibit or destroy microbial growth in the infected host. They are members of an extremely diverse group of metabolic products known as secondary metabolites, which are complex organic molecules that are not essential for normal cell growth and reproduction and are produced only after an organism has established itself in its environment. The ideal antibiotics to treat an infection must be readily available, inexpensive, chemically stable, easily administered, non-toxic and non-allergenic to human (Bauman, 2004; Cowan & Talaro, 2009). There are five main categories of antibiotics according to their mechanisms of action: inhibit cell wall synthesis, inhibit protein synthesis, alter cell membranes, anti metabolites and inhibit nucleic acid synthesis. To ascertain the efficacy of antibiotics, diffusion susceptibility tests also known as *Kirby–Bauer* technique, is an agar diffusion test that provides useful data on antimicrobial susceptibility. This method was widely used on the determination of antimicrobial resistance of the bacteria isolated from food or hands of food handlers (Akond, Alam, Hassan, & Shirin, 2009; Albuquerque, Macrae, Sousa, Vieira, & Vieira, 2007; Harakeh et al., 2005; Miranda et al., 2009; Normanno et al., 2007; Pesavento, Ducci, Comodo, & Nostro, 2007; Sasidharan, Prema, & Yoga Latha, 2011). In Tan, Bakar, Abdul Karim, Lee, and Mahyudin (2013) report on food hygiene practices by food handlers in Malaysia that the least practiced habits were hand washing and the usage of face mask during food preparation. This would facilitate the transmission of bacteria such as *Staphylococcus aureus* into food, and due to the different background of food handlers, carriers of multiple resistant *S. aureus* can contribute to the widespread of antibiotic resistance of *S. aureus*. In community-acquired infections,

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Escherichia coli and *S. aureus* are the most frequently isolated bacteria (Thibaut et al., 2010).

As more bacteria become resistant to traditional antibiotics, this leads to emergence and re-emergence of multidrug-resistant pathogens (Abulreesh & Organji, 2011; Acco, Ferreira, Henriques, & Tondo, 2003; Akond et al., 2009; Albuquerque et al., 2007; Lei et al., 2010; Platell, Johnson, Cobbold, & Trott, 2011; Simeoni et al., 2008). This study was performed to determine the antimicrobial resistance of *E. coli* and *S. aureus* isolates from food handlers' hands at primary schools in Hulu Langat district, Selangor (Malaysia). Results of this study may be applicable as useful information related to multidrug resistant bacteria found on food handlers' hands.

2. Materials and methods

2.1. Sampling and culturing procedures

A total of 1020 samples were collected from 85 food handlers' hands at 38 primary schools in Hulu Langat district. Microbiological analysis was conducted on food handlers' hands to test for APC (Aerobic Plate Count), *E. coli*/coliform and *S. aureus* counts. Samples were collected from the right and left palms of the food handlers in duplicate. The collection was done during weekdays (06:00–10:00) at the following intervals; before, during and after preparation of ready-to-eat (RTE) foods such as 'nasi lemak', sandwiches, fried foods and burgers.

Sterile swabs (Premier, China) were removed from pre-coded test tubes that contained 5 ml of 1 × sterile phosphate-buffered saline with pH 7.4 ± 1 (Oxoid, Basingstoke, UK) and the targeted areas (palms of food handlers) were swabbed. Sampling was performed by swabbing the areas horizontally, vertically and diagonally by using aluminum templates with the size of 2 cm × 5 cm. The whole procedures were done aseptically to minimize the risk of contamination. Swabs were then placed back into the pre-coded test tubes. The collected samples were stored and transported in insulated boxes filled with crushed ice prior to analysis. The storage temperature was within 0–4 °C while the transport duration to the laboratory was within 15 min to 1 h. Analyses were performed immediately upon arrival to the laboratory.

Each of the food handlers' palms was analyzed for APC, *E. coli*/coliform and *S. aureus* counts by using Petrifilm APC, Petrifilm *E. coli*/coliform and Petrifilm STX Count Plates respectively (3M Microbiology, St. Paul, USA). Swab contact method on Petrifilm plates was used to evaluate APC, *E. coli*/coliform and *S. aureus* counts on food handlers' palms (Evancho, Sveum, Moberg, & Frank, 2001).

2.2. Isolation and identification of E. coli and S. aureus

Single colony of *E. coli* and *S. aureus* for each sample from the food handlers' hands were isolated stored at −20 °C until further examinations (Baldwin, Ziegler, Green, & Thomas, 2000; Portle, 2009; StockingerLab, 2001). The colonies were identified through a series of biochemical (physiological) tests as described in Bergey and Holt (1994). Before the biochemical tests were held, the isolates were revived on nutrient agar to obtain fresh cultures. Gram staining, catalase test and mannitol salt agar were used to identify suspected *S. aureus* isolates while Gram staining, oxidase test, lactose fermentation, indole and citrate tests were used to identify presumptive *E. coli* isolates. In addition, culture characteristics of the bacteria were also observed.

2.3. Antimicrobial disc susceptibility tests

Disc diffusion methods were used to examine the antimicrobial resistance of *S. aureus* and *E. coli* isolates on sterile Mueller–Hinton agar (Wikler, 2006). Ten types of antibiotic discs with different concentrations were used. The antibiotic discs used were Penicillin (10 μg), Ampicillin (10 μg), Gentamicin (10 μg), Kanamycin (30 μg), Tetracycline (30 μg), Chloramphenicol (30 μg), Trimethoprim (5 μg), Sulfamethoxazole-Trimethoprim (25 μg), Ciprofloxacin (5 μg) and Nitrofurantoin (300 μg). These antibiotics were chosen based on their different molecular structures and modes of action and must be effective against both Gram-negative and Gram-positive infections. The diameters of zones were measured to the nearest whole millimeter and the bacteria were categorized into resistant, intermediate and susceptible as mentioned in Wikler (2007). CLSI 2006 shows the disc diffusion methods whereas CLSI 2007 shows the susceptibility of bacteria (resistant, intermediate or susceptible).

2.4. Statistical analysis

Statistical analyses were performed using SPSS Statistics version 19. Frequencies and percentages were calculated for all variables as appropriate.

3. Results and discussion

3.1. Prevalence of E. coli and S. aureus on food handlers' hands

Table 1 displays the prevalence of *E. coli* and *S. aureus* on 85 food handlers' hands before, during and after RTE foods preparation. The results showed that the prevalence of *S. aureus* (65.88–74.12%) was significantly higher ($P < 0.05$) than the prevalence of *E. coli* (9.41–14.12%). The high prevalence of *S. aureus* among the food handlers may indicate that they did not maintain good personal hygiene when handling RTE foods. It also may be due to the contamination introduced by food handlers through skin lesions or by sneezing or coughing (Bischoff, Wallis, Tucker, Rebouyssin, Pfaller, Hayden & Sherertz, 2006). As hygiene practices reported by Tan et al. (2013), hand washing practices and face mask are not commonly used by food handlers may cause the higher prevalence of *S. aureus* from the hands. Fortunately, the prevalence of *E. coli* was quite low compared to *S. aureus*. For *E. coli*, they existed most often after the preparation of RTE foods (14.12%) and they was the same (9.41%) for both before and during the preparation of RTE foods whereas for *S. aureus*, they existed most frequently before RTE foods preparation (74.12%)and reduced after (70.59%) and during (65.88%) the preparation of RTE foods. Statistical analyses found there were no significant ($P > 0.05$) difference in the mean log cfu/cm^2 for *E. coli* , *S. aureus* and APC on the food handlers' hands for the three intervals of food preparation. The increased occurrence of *E. coli* after food preparation may also due to the cross contamination of the food to the food handlers' hands. From observation, some food

Table 1
Mean of *E. coli* and *S. aureus* on 85 food handlers' hands before, during and after RTE foods preparation.

Intervals	*E. coli*		*S. aureus*		Statistical difference ($P < 0.05$)
	[a]Mean ($\log_{10}$ CFU/cm^2)	Percentage (%)	Mean ± standard deviation ($\log_{10}$ CFU/cm^2)	Percentage (%)	
Before	0.2[b] ± 0.4	9.41	0.5[a] ± 0.7	74.12	$F = 8.855$; $P = 0.003$
During	0.2 ± 0.4	9.41	0.3[a] ± 0.5	65.88	$F = 3.412$; $P = 0.066$
After	0.2[b] ± 0.4	14.12	0.4[a] ± 0.7	70.59	$F = 5.317$, $P = 0.022$

[a] Data were reported as mean ± standard deviation for four replicates ($\log_{10}$ CFU/cm^2).
[b] Significant difference between *E. coli* and *S. aureus*.