Original Article

Prevalence of *Escherichia Coli* O157:H7 and *Enterobacteriaceae* on Hands of Workers in Halal Cattle Abattoirs in Peninsular Malaysia

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Abstract

Background: **Several occupational diseases of multiple origins are encountered among abattoir workers. Presence of indicator microorganisms (coliforms) on hands of workers can be used a gauge for hygienic practices.**

Methods: **A cross-sectional study was performed to assess the prevalence of *E.coli* and *enterobacteriaceae* among Halal abattoir workers in some government halal abattoirs of Malaysia. A total of one hundred and sixty-five hand swab samples were collected from workers of Halal abattoirs in Malaysia. The samples were subjected to microbiological analysis for characterisation and serotyping.**

Results: **The results have shown that no *Escherichia coli* O157:H7 was isolated on the hands of abattoir workers before and after work. However, a total prevalence of 9.7% was recorded for all samples during work. For non-O157:H7, total prevalence of 33.3% during work and 13% after work were obtained. High prevalence was recorded in sample taken during work from Tampin, Jasin and Kemaman (100% each) while low prevalence where observed in Shah Alam, Banting and Ipoh (20% each).**

Conclusions: **Based on the findings the hygienic practices of hand washing among the workers in few locations was found to be low especially after work.**

Keywords: *Escherichia coli O157:H7, occupational safety, foodborne disease, zoonoses, public health, prevalence*

Introduction

An abattoir is a place approved by a relevant authority and registered for the hygienic slaughtering and inspection of animals. Generally, the permissions include processing and efficient operative procedures to store and preserve meat products in accordance with specified guidelines for human consumption (1). Due to an increase in population and urbanisation, the slaughter house can serve as a place where pathogenic organisms grow and proliferate, depending on the general hygienic practices and preventive measures applied by

the abattoir. Microorganisms of different species such as yeasts and moulds are implicated in the spoilage and contamination of meat and its products. Inappropriate hygienic practices by the abattoir workers often lead to cross contamination of the cattle carcasses. Other sources associated with meat contamination include using contaminated water for washing carcasses, air particles in the dressing, cooling, and cutting rooms or tables and the environment (2). Malaysia is located in a tropical region where the average temperature of its environment can enhance bacterial growth. Countries within the tropics are prone to various cases of epidemics especially in those areas with insufficient potable water and poor handling practices by the abattoirs. This may result in heavy microbial contamination that predisposes the meat to rapid deterioration and, consequently, results in food poisoning (3). Worker mishandling of food is one of the main causes of food borne disease outbreaks. Microbes, including Salmonella spp., Escherichia coli and Clostridium spp., are among the bacteria that are of global public health concern because of the role they play in meat contamination and food-borne disease transmission. Many studies have reported that certain strains of these organisms are resistant to various antimicrobials (4, 5, 6, 3, 7).

In the abattoir industry, occupational zoonotic diseases from different sources are encountered by workers who usually handle the slaughter of various species of animals for human consumption, due to their close contact with animals during slaughtering and processing. Consequently, abattoir workers are one of the main groups at risk for contracting occupational zoonosis. Symptoms of zoonotic diseases may be noticed easily, but conclusions about the disease require standard laboratory diagnostic procedures including microbiological, immunological and molecular techniques (8). Zoonotic diseases are defined as those infections and diseases which are naturally transferred from animals to humans. Recent estimates show that 70% of emerging infectious diseases are zoonotic (9). In addition, over 300 zoonotic diseases of multiple aetiologies have been recorded and linked with high morbidity and mortality (10). The diseases occur in both males and females, in all seasons, in all climatic zones, in all age groups and in urban and rural locations (10, 11).

A global population increase coupled with a high demand for meat and meat products have made human contact with animals inevitable. Transportation of animals across international borders to augment local stock can increase the risk of zoonotic diseases especially when those animals are imported from endemic zones (12). Zoonotic diseases can be disseminated via numerous routes (13). However, the most common port of entry for the microbe is direct contact with the animals by the employees working in the abattoirs (14, 10, 11, 15).

Enterohaemorrhagic *Escherichia coli* (EHEC) is one of the most common zoonotic agents that can be transmitted from animals to humans and is a cause of severe disease and mortality in outbreaks associated with foods (16). An *E. coli* O157:H7 serotype that was implicated in an outbreak of haemorrhagic colitis in the United States produced Shiga toxin (17). In several outbreaks, *Escherichia coli* that produced Shiga toxin were epidemiologically associated with haemolytic uremic syndrome (18). Under normal circumstances, generic *E.coli* is a harmless member of the normal flora in humans and animals. However, by various methods, the bacteria may become pathogenic by acquiring virulence genes that confer pathogenicity to it. The World Health Organization (WHO) reported that illnesses related to the ingestion of contaminated foods (such as meat) are some of the most common public health problems in the modern world (19). These illnesses cause a decrease in manpower, which invariably results in considerable economic loss. Ground beef is responsible for 75% of *E.coli* O157:H7 outbreaks (20). Cattle faeces can directly contaminate dairy products and undercooked minced beef during the milking and slaughtering processes (21). The first line of action to ensure the safety of workers and meat in the abattoir is to avoid cross contamination during carcass processing. Hand washing is one of the major steps to reducing the public health burden that can arise from the abattoir. The aim of this study is to assess the hygienic practice of hand washing among halal abattoir workers.

Materials and Methods

Study location

Malaysia consists of 13 states. Six states were randomly selected for the intervention: Pahang, Selangor, Terengganu, Negeri Sembilan, Melaka and Perak. The halal abattoirs visited were Tampin, Shah Alam, Senawang, Kuala Pilah, Banting, Kuantan, Kemaman, Dungun, Jasin, Ipoh and Teluk Intan (Figure 1).

Figure 1. Locations of the abattoirs that were sampled throughout Malaysia

Sampling

Population: Butchers from government halal cattle abattoirs, including part-time workers, were selected for the study.

Sampling frame: All butchers working in government halal cattle abattoirs.

Sample collection: Fifty-two abattoir workers participated in the study and a total of 165 samples were collected from the hands of workers before, during, and after work. Sterile cotton swabs that contained transport medium were used for sample collection. Samples from the hands of abattoir workers were collected before and after work. All samples were stored in a cooler box and transported to the Applied Microbiology Laboratory at the Universiti Putra Malaysia.

Bacteriological Analysis

All samples were subjected to bacteriological analysis. The samples were inoculated onto Chromocult© Agar and MacConkey Sorbitol Agar (CT-SMAC) (Merck, Darmstadt, Germany) agar for the isolation of *Escherichia coli* O157:H7 and other *enterobacteriaceae*. Samples were collected by swabbing both hands using a sterile swab stick after hand washing but before the workers embarked on cattle processing. In addition, samples were also collected during carcass processing and at the completion of the day's work. In this manner, the prevalence of *E.coli* was determined during three stages of work.

Laboratory procedures

The swabbed samples were appropriately inoculated onto Chromocult® Agar (Merck, Germany). Colonies with dark blue to violet coloration were counted after incubation at 37°C for 24 h. All isolates that showed dark blue coloration on Chromocult® Agar were characterised biochemically based on methods previously described (22). Isolates identified biochemically as *E.coli* were further screened on Cefixime Tellurite Sorbitol MacConkey agar (Merck, Germany) by incubation for 24 h at 37°C. *E.coli* O157:H7 appeared colourless, while non-O157:H7 appeared pink (23). Colonies that appeared colourless (non-sorbitol fermenters) on CT-SMAC were presumptively identified as *E.coli* O157:H7 and were preserved on a nutrient agar slant for confirmation using the slide agglutination test. Isolates that were colourless

were serotyped using a Serotest® for *E.coli* O157:H7 (S&A Lab., Thailand), a polyclonal antibody produced for serological identification based on the agglutination method. A drop of serum was placed onto the test area and a drop of saline was placed onto the control area of a clean glass slide. Using a platinum wire loop, a portion of the bacterial growth was transferred onto the drop of serum and mixed. Another portion of the growth was mixed with normal saline solution (control). The glass slide was tilted back and forth for one minute. Agglutination was observed and recorded according to the manufacturer's instructions.

Results and Discussion

No *Escherichia coli* O157:H7 were isolated from the hands of abattoir workers before and after work. However, a total prevalence of 9.7% was recorded for all samples during work. Only workers from two abattoirs, Kuala Pilah (67%) and Ipoh (20%), were found to have *E.coli* O157:H7 on their hands while working as shown in Table 1. As shown in Table 2, there was a total non-O157:H7 prevalence of 33.3% during work and 13% after work for all abattoirs. The occurrence of the bacteria during work may be linked to contact with the intestinal contents or hides of the animals.

None of the abattoir workers had non-O157:H7 *E.coli* hand contamination before work. The absence of the bacteria before work may arise from hand washing preformed prior to animal processing. A high prevalence was recorded in samples collected from workers' hands during work in the Tampin, Jasin and Kemaman (100% each) abattoirs while a low prevalence was observed in samples from the Shah Alam, Banting and Ipoh abattoirs. The presence of the pathogens on workers' hands occurred as a result of contact with cattle faecal matter during animal processing. Tan et al. (24) reported that the prevalence of *E.coli* among food handlers was 71.76%, 71.76% and 68.24% for hand swabs collected before, during and after work. However, a lower prevalence (24%) was reported by Mayada et al. (25). Other pathogenic bacteria isolated from the swab samples include *Salmonella enteritidis* and *Citrobacter freundii*. The prevalence of the bacteria ranges from 20–100% during working hours while after work it was 50% to 100% (Table 3).

In addition to *E.coli* and *Salmonella enteritidis*, the sample showed that *Citrobacter freundii* was found to be at a higher prevalence range (60%–100%) among the abattoir workers during work. A lower prevalence of *Citrobacter freundii* was recorded (20–40%) after work compared to *S. enteritidis* (Table 4).

Table 1. Prevalence of *E.coli* O157:H7 on the hands of workers based on abattoir location

Location	Before work (n = 52)	During work (n = 52)	After work (n = 52)
Shah Alam	(0/5)0%	0%	0%
Banting	(0/50%	0%	0%
Senawang	(0/5)0%	0%	0%
Kuala Pilah	(0/6)0%	67%	0%
Tampin	(0/5)0%	0%	0%
Jasin	(0/5)0%	0%	0%
Ipoh	(0/5)0%	20%	0%
Teluk Intan	(0/5)0%	0%	0%
Kuantan	(0/4)0%	0%	0%
Kemaman	(0/4)0%	0%	0%
Dungun	(0/3)0%	0%	0%

Prevalence before work = 0%, during work = 9.7%, after work = 0%

Table 2. Prevalence of non-O157:H7 on the hands of workers based on abattoir location

Location	Before work (n = 52)	During work (n = 52)	After work (n = 52)
Shah Alam	(0/5) 0%	(1/5) 20%	(0/5) 0%
Banting	(0/5) 0%	(1/5) 20%	(0/5) 0%
Senawang	(0/5) 0%	(1/5) 0%	(0/5) 0%
Kuala Pilah	(0/6) 0%	(4/6) 67%	(2/6) 33%
Tampin	(0/5) 0%	(5/5) 100%	(0/5) 0%
Jasin	(0/5) 0%	(5/5) 100%	(5/5) 100%
Ipoh	(0/5) 0%	(1/5) 20%	(1/5) 20%
Teluk Intan	(0/5) 0%	(0/5) 0%	(1/5) 20%
Kuantan	(0/4) 0%	0/4) 0%	(0/4) 0%
Kemaman	(0/4) 0%	(4/4) 100%	(0/4) 0%
Dungun	(0/3) 0%	(0/3) 0%	(0/3) 0%

Total prevalence before (**0%**), during (**35.5%**), and after (**13%**) work

Table 3: Prevalence of S entridis on hand swabs

Location	Before work (n = 52)	During work (n = 52)	After work (n = 52)
Shah Alam	(0/5) 0%	(1/5) 20%	(3/5) 60%
Banting	(0/5) 0%	(2/5) 40%	(0/5) 0%
Senawang	(0/5) 0%	(0/5) 0%	(3/5) 60%
Kuala Pilah	(0/6) 0%	(6/6) 100%	(4/6) 67%
Tampin	(0/5) 0%	(5/5) 100%	(5/5) 100%
Jasin	(0/5) 0%	(5/5)100%	(5/5) 100%
Ipoh	(0/5) 0%	(2/5) 20%	(0/5) 0%
Teluk Intan	(0/5) 0%	(0/5) 0%	(2/5) 40%
Kuantan	(0/4) 0%	(0/5) 0%	(5/5) 100%
Kemaman	(0/4) 0%	(4/4) 100%	(0/4) 0%
Dungun	(0/3) 0%	(3/3) 100%	(0/3) 0%

Table 4: Prevalence of C. freundii on hand swabs

Location	Before work (n = 52)	During work (n = 52)	After work (n = 52)
Shah Alam	(0/5) 0%	(0/5) 0%	(2/5) 40%
Banting	(0/5) 0%	(3/5) 60%	(0/5) 0%
Senawang	(0/5) 0%	0/5) 0%	(2/5) 20%
Kuala Pilah	(0/6) 0%	(4/6) 67%	(2/6) 33%
Tampin	(0/5) 0%	(0/5) 0%	(0/5) 0%
Jasin	(0/5) 0%	(0/5) 0%	(0/5) 0%
Ipoh	(0/5) 0%	(0/5) 0%	(0/5) 0%
Teluk Intan	(0/5) 0%	(0/5) 0%	(0/5) 0%
Kuantan	(0/4) 0%	(0/4) 0%	(0/4) 0%
Kemaman	(0/4) 0%	(4/4) 100%	(0/4) 0%
Dungun	(0/3) 0%	(3/3) 100%	(0/3) 0%

Conclusions

The prevalence of *E.coli* among the workers varied based on the stage of work. However, the absence of pathogens on their hands before work shows that potential contamination of cattle carcass from external sources is almost unlikely. Workers harbouring non-O157:H7 may serve as a potential source of contamination and infections. The presence of *E.coli* O157:H7 during work is a public health concern due to the possibility of cross-contamination between the hands of workers and the animal carcasses. Further studies, using molecular techniques, need to be conducted to enumerate the number of bacteria in each sample and to characterise the bacteria.

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Ethical Approval

This study had been approved by Ethics Committee for Research Involving Human Subject (JKEUPM). Ref: UPM/TNCPI/RMC/1.4.18.1(JKEUPM)/F2

Conflict of Interests

None

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Authors' Contributions

Drafting of the article: AMT
Critical revision of the article for important intellectual content: SBMT
Final approval of the article: SBMT, MNMD, SKB
Provision of study materials or patients: MNMD, SKB
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