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A STUDY OF CROSS-CONTAMINATION OF FOODBORNE PATHOGENS ON THE KITCHEN SURFACES

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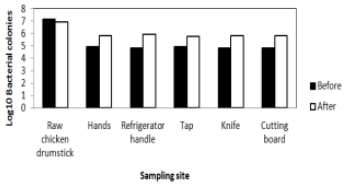
Graphical abstract



Abstract

Cross-contamination provides the opportunity for various of bacteria to be deposited on each of the surface contact during meal preparation. Raw poultry especially raw chicken was the main reservoir of foodborne pathogens that can cause foodborne diseases. Therefore, a study on the potential of cross-contamination contribute to spread E. coli, Salmonella spp. and S. aureus on the kitchen surfaces during chicken preparation was conducted. A total of 36 isolates were collected from six sampling sites before and after the chicken preparation. The enumeration of the bacteria from the sampling sites showed a significant change in the mean total plate counts (TPC) of the isolates before and after the chicken preparation. These results emphasized that cross-contamination occurred around the sampling sites during the preparation of the chicken. Isolation and identification of the three foodborne pathogens, *E. coli*, *Salmonella* spp. and *S. aureus* were carried out on its respectively selective and differential media. The presumptive identified foodborne pathogens were confirmed as *E. coli*, *Salmonella* spp. and *S. aureus* according to their microscopic and biochemical characteristics.

Keywords: Cross-contamination, foodborne pathogens, kitchen surfaces

Abstrak

Pencemaran silang memberi peluang kepada pelbagai bakteria untuk dihantar kepada setiap permukaan bersentuhansemasa penyediaan makanan. Daging mentah terutama ayam mentah adalah takungan utama patogen bawaan makananyang boleh menyebabkan penyakit bawaan makanan. Oleh itu, kajian mengenai potensi pencemaran silang menyumbang untuk menyebarkan *E.coli*, *Salmonella* spp. Dan *S.aureus* pada permukaan dapur semasa penyediaan ayam telah dijalankan. Sebanyak 36 pencilan telah dikumpulkan daripada enam kawasan persampelan sebelum dan selepas penyediaan ayam. Penghitungan bakteria dari kawasan persampelan menunjukkan perubahan yang signifikan dalam min jumlah kiraan plat (TPC) daripada pencilan sebelum dan selepas penyediaan ayam. Keputusan ini menekankan bahawa pencemaran silang berlaku di sekitar kawasan persampelan semasa penyediaan ayam. Pengasingan dan pengenal pastian tiga patogen bawaan makanan, *E.coli*, *Salmonella* spp. Dan *S.aureus* telah dijalankan ke atas media selektif dan pembezaan masing-masing. Andaian patogen bawaan makanan yang dikenalpasti telah disahkan sebagai *E.coli*, *Salmonella* spp. Dan *S.aureus* mengikut ciri-ciri mikroskopik dan biokimia mereka.

Kata kunci: Pencemaran silang, patogen bawaan makanan, permukaan dapur

1.0 INTRODUCTION

Foodborne diseases are a widespread public health problem. There are several major factors contribute to the illnesses, which one of the major factors is the poor hygiene practices [1]. Poor food handling and sanitation lead into cross-contamination and contribute to the transmission of foodborne diseases. Cross-contamination occurs when pathogenic bacteria from a source directly or indirectly transferred to other foods or objects. Contact surfaces are one of the important sources of pathogens via cross-contamination [2], [3]. The pathogenic bacteria can be easily transferred from raw materials to the kitchen surfaces during the food preparation. Colonization of pathogenic bacteria in kitchen utensils, surfaces and the cross-contamination of the bacteria between humans and the kitchen environment can impact human health. The cross-contamination between hands and various kitchen utensils has been proven to spread various pathogenic bacteria including *E. coli, S. aureus,* and *Salmonella* spp. [4]-[8].

2.0 EXPERIMENTAL

The raw chicken drumstick was purchased randomly from the local market and transported to the laboratory. Immediately after reaching the laboratory, the raw chicken drumstick was transferred and stored in the refrigerator. After half an hour, the raw chicken drumstick was removed from the refrigerator and washed with the tap water in the tap. The raw chicken drumstick then was placed on the cutting board before cuts into small pieces using a knife. A swab approximately 10 cm^2 from totaled 6 sites, including the raw chicken drumstick and five sites comprising the person's hand, refrigerator handle, tap, knife and cutting board were taken before and immediately after the chicken preparation, by using one swab moistened with Buffered Peptone Water (BPW). The dilution of BPW was used to recover microorganism from the swabs to determine the total plate count (TPC). Dilutions for the chicken samples were 10^{-1} - 10^{-4} while the dilution of the samples taken before chicken preparation were 10^{-1} - 10^{-2} and samples taken after chicken preparation were 10^{-1} - 10^{-3}. 0.1 mL from each dilution from the sample was spread on the nutrient agar and incubated at 30 °C for 48±3 h. After the incubation, the number of Colony Forming Units (CFU) on the plates for each dilution was counted. Typically numbers between 30 and 300 are considered to be in the range is considered statistically significant. If the number of CFU on the plate are greater than 300, the CFU was recorded as too numerous to count (TNTC).

The presence of *E. coli*, *Salmonella* spp. and *S. aureus* in the isolates was determined by culturing the isolates into specific enrichment media followed by plate on specific selective and differential media (Table 1). The plate was then incubated at 37 °C for 24 h.

Table 1 Specific enrichment media, selective and differential media for identification of *E. coli*, *Salmonella* spp. and *S. aureus*

Foodborne pathogen	Enrichment media	Selective and differential media
E. coli	Lactose Broth	Eosin Methylene Blue (EMB) Agar MacConkey (MAC) Agar
Salmonella spp.	Selenite Cystine (SC) Broth	Hektoen Enteric (HE) Agar Xylose Lysine Deoxycholate (XLD) Agar
S. aureus	Brain-Heart Infusion (BHI) Broth	Baird-Parker Agar (BPA) Mannitol Salt Agar (MSA)

For sub-culturing, five presumptive identified colonies of *E. coli, Salmonella* spp. and *S. aureus* grown on the specific selective and differential media were picked and inoculated on nutrient agar. The plate then incubated at 37°C for 24 h before use in confirmatory test. After proper incubation, five pure colonies of *E. coli, Salmonella* spp. and *S. aureus* were selected for confirmatory tests. Confirmatory tests which involved preliminary test (Gram staining) and a series of biochemical tests (catalase test, oxidase test, citrate test, motility test, TSI agar test, Indole test, OF-glucose test, urease test, methyl red test, starch hydrolysis) were performed to confirm the presence of the *E. coli*, *Salmonella* spp. and *S. aureus* in the isolates from the sampling sites.

3.0 RESULTS AND DISCUSSION

Only the mean total plate counts colonies of the raw chicken drumstick showed a decreasing in numbers after the chicken preparation. Isolates from other sampling sites showed an increase in the CFU/mL after chicken preparation compared to those sampled before the chicken preparation (Figure 1). This indicated the occurrence of cross-contamination from

chicken to sites and from one site to another site during the chicken preparation.

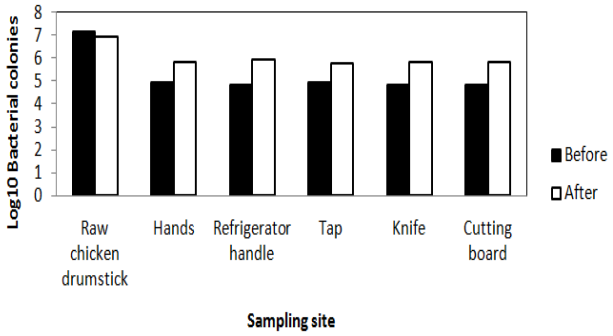


Figure 1 Number of bacteria isolated from the sampling sites before and after the chicken preparation

Among the sites sampled before the chicken preparation, hand was found to be the source for the second highest number of bacteria after raw chicken drumstick followed by tap, refrigerator handle, knife and cutting board (Figure 1). After the chicken preparation, the highest number of bacteria was found on the raw chicken drumstick followed by refrigerator handle, cutting board, hand, knife and tap. This showed indirect evidence of cross-contamination because generally only hands will contact from one site to another during meal preparation. Several studies reported that various bacteria, including *E. coli, S. aureus,* and *Salmonella* spp. can survive for hours or days on hands and utensils after initial contact with the contaminated raw poultry [9]-[11]. Therefore, it had been suggested that the best way to minimize the cross-contamination in the kitchen during food preparation was to implement hygienic practices before and after the preparation of the meal and cook the raw poultry thoroughly with appropriate temperature [12].

Positive colonies (+) grew on the media indicated that the isolates have presumptive identified colonies of *E. coli*, *Salmonella* spp. and *S. aureus* (Table 2). Positive colonies of *E. coli* grown as blue-black with a green metallic sheen colonies on the EMB agar plates and grown as red-pink colonies on MAC agar plates. Meanwhile, *Salmonella* spp. grown as completely blue-green or black colonies on HE agar plates and grown as red colonies on XLD agar plates. *S. aureus* grew as black or grey shining colonies surrounded by an opaque zones on BPA plates and grown as yellow coloured colonies surrounded by yellow zones on MSA plates.

Table 2 Overall identification results of *E. coli*, *Salmonella* spp. and *S. aureus* colonies on the specific selective and differential media

Sampling site	Before			After		
	EMB agar	HE agar	BPA	MAC agar	XLD agar	MSA
Raw chicken drumstick	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]
Hand	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]
Refrigerator handle	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]
Tap	+[a]	-[b]	-[b]	+[a]	+[a]	+[a]
Knife	-[b]	-[b]	-[b]	+[a]	+[a]	+[a]
Cutting board	-[b]	-[b]	-[b]	+[a]	+[a]	+[a]

Before the chicken preparation, only raw chicken drumstick, hand and refrigerator positive for *Salmonella* spp. and *S. aureus*. In contrast, *E. coli* was found on all sampling sites except on the knife and cutting board. However, after the chicken preparation, *E. coli*, *Salmonella* spp. and *S. aureus* were found in all the sampling sites (Table 2).

The presumptive identified colonies of *E. coli*, *Salmonella* and *S. aureus* showed consistent results on the confirmatory tests before and after the chicken preparations (Table 3). The confirmatory test results obtained were identical to the typical strains reported results [13]-[22].

4.0 CONCLUSION

The increases in the CFU/mL and the number of bacteria (Log_{10}) after the chicken preparation emphasized that cross-contamination occurred from one site to another site during the preparation of the chicken. The identical results of the confirmatory tests of the presumptive identified colonies of with its typical strains indicated that the presumptive identified colonies were positive for *E. coli*, *Salmonella* spp. and *S. aureus*.

Table 3 Summary of the confirmatory tests results tested on the all presumptive identified colonies of *E. coli*, *Salmonella* spp. and *S. aureus* isolated from the sampling sites before and after the chicken preparation

Confirmatory tests	*E. coli*	*Salmonella* spp.	*S. aureus*
Gram's staining	Colour: Pink Shape: Rod Grams : Negative	Colour: Pink Shape: Rod Grams :Negative	Colour: Purple Shape: Cocci Grams : Positive
Catalase test	+[c]	+[c]	+[c]
Oxidase test	-[d]	-[d]	-[d]
Citrate test	-[d]	+[c]	-[d]
Motility test	+[c]	+[c]	-[d]
TSI agar test	Reaction: A/A[e] Gas: Yes H_2S: None	Reaction: K/A[f] Gas: Yes H_2S: Yes	Reaction: A/A[e] Gas: None H_2S: None
Indole test	+[c]	-[d]	-[d]
OF-glucose test	+[c]	+[c]	+[c]
Nitrate reduction test	+[c]	+[c]	+[c]
Urease test	-[d]	-[d]	+[c]
Methyl red test	+[c]	+[c]	+[c]
Starch hydrolysis	+[c]	-[d]	-[d]

[c] (+) indicate positive reaction
[d] (-) indicate negative reaction
[e] A/A represent acid over acid reaction
[f] K/A represent alkali over acid reaction

As a recommendation, further investigations and confirmatory tests are needed to increase the accuracy especially on the level of identification of the foodborne pathogens. Molecular analysis as an identification of the foodborne pathogens could be a more accurate alternative or as an addition to the methods already performed. Immuno-magnetic separation and real-time polymerase chain reaction (IMS-RT-PCR) method may be applied for accurate confirmation of the foodborne pathogens. This method is more accurate and suitable for rapid detection of the foodborne pathogens. Conjugation of genotypic approaches in antimicrobial susceptibility testing can provide more sensitive, reliable and accurate information in identifying the bacteria responses towards certain antimicrobial. Rapid development of DNA-based assay and phenotypic analysis offer promises of increased efficiency in the detection of bacteria resistance at the genetic level.

Maintaining optimal hygiene practices are very important in food preparation. Food preparer should have a good knowledge in hygienic practices to ensure that the food consumed by the consumers are clean and safe. Implementation of good hygienic practices can minimize the risk of cross-contamination of foodborne pathogens. Cross-contamination of foodborne pathogens can cause serious illness that can bring to death. Therefore, hygienic practice by the food preparer must not be neglected. Good hygienic practices in food preparation involve properly washing hands with soap and warm water prior to cooking or after handling raw foods, correct cooking and storage temperature, and proper cleaning and sanitizing areas and kitchen utensils prior to contact with raw food and ready-to-eat food.

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