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OCCURRENCE AND ANTIBIOTIC RESISTANCE OF *Salmonella* sp. IN SMOKED PRODUCTS FROM LOCAL STREET STALLS AROUND KUALA PILAH, NEGERI SEMBILAN

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Abstract

Smoked food was one of the most authentic dishes in Malaysian cuisines. However, local consumers were still unaware on the hygienic level of these smoked products. Nowadays, the smoked products were smoked in an open space that allowed the contamination of bacteria on the food. This study aimed to determine the occurrence and antibiotic resistance of *Salmonella* in the smoke catfish and meat at the local street stalls in Kuala Pilah, Negeri Sembilan by MPN-PCR methods. The microbial concentration of *Salmonella* sp. in smoked catfish was 2.4 x 10^{-8} MPN/g in smoked catfish and 2.9 x 10^{-7} MPN/g in smoked meat and were confirmed by culturing on selective agar of Salmonella Shigella agar. The prevalence of *Salmonella* sp. was found to be 68% in both samples by MPN-PCR approach. *Salmonella* sp. were 100% detected in smoked catfish followed by smoked meat by 44% respectively. All the positive isolates from MPN-PCR were continued with antibiotic susceptibility to determine the resistance level of *Salmonella* sp. towards selected antibiotics. As result, the isolates showed a multi-resistance patters from one to four antibiotics tested with MAR indices ranging from 0.25 to 1.00. The outcome indicated a high rate of foodborne pathogens which indicated the need to create the awareness on the safety and proper handling of smoked products to minimization of any potential health hazard caused by this foodborne pathogen.

Keywords: *Salmonella* sp., smoked foods, MPN-PCR, Antibiotic Susceptibility, MAR index

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Introduction

Foodborne disease such as cholera, typhoid fever, hepatitis A, dysentery and food poisoning (Blackburn and McClure, 2002) usually are caused by microorganism like bacteria, virus and parasite (Abdul-Mutalib *et al.*, 2015). In Malaysia, 5000 cases have been reported with food poisoning and typhoid in 2013 (MOH, 2013). Most of the foodborne diseases in Malaysia are associated with food handler's insanitary practices during food manipulation (Soon *at al.,* 2011) which pathogenic bacteria may transfer to food items and cause illnesses (Abdul-Mutalib, 2015). The contamination of bacteria to food items also influenced by several factors such as temperature, humidity, and interaction between microorganism and the food (Hamad, 2012).

Smoked foods are among the most authentic dishes in Malaysia. The smoking process itself were done in an open space might contribute to the contamination of the microorganisms on the foods. The complex composition of the smoke with higher contents of carbon and organic particle might be a main nutrient source for the microorganisms to survive. Moreover, the smoking process of using heat radiation from the fire to cook the foods might create a favourable temperature for the microorganisms to grow especially the pathogenic ones. *Salmonella* sp. is one of the most pathogenic bacteria in food which were generally identified as gram-negative rod-shaped bacteria, ranging from 0.7 to 1.5 x 2 to 5 µm in size, non-fastidious,

grow at temperature range of 5 to 47ºC with optimum temperature of 35 – 37ºC and often killed at 70°C (Pui *et al.*, 2011).

Salmonella sp. is quite complex to study because it has a numerous system in classifying them into species, subspecies and serotypes which differentiate and determine their virulence. The detection and isolation of *Salmonella* sp. in food samples is important in order to identify whether the food are risks to the population or consumable. The MPN-PCR is an effective tool to simultaneously detect the occurrence of foodborne pathogens quantitatively and qualitatively.

Methods

Sample Collection

The samples of smoked meat and smoked catfish were purchased randomly from stalls along the road from Kuala Pilah to Seremban, Negeri Sembilan.

MPN-PCR Method

A 10g of food samples were aseptically weighed and transferred into sterile stomacher bag and being pummeled in stomacher for 120s with 90 mL of buffered peptone water (BPW; Merck Darmstadt, Germany). A three-tube most probable number (MPN) serial dilution were done up to 10^{-3}. Each fold was conducted in triplicate. One ml from each dilution were transferred into three microcentrifuge tubes. All the tubes were incubated at 37°C for 18 - 24 hours. After incubation, all turbid MPN tubes were subjected to DNA extraction by modified boiled cell method (Chai *et al.*, 2007; Tang *et al.*, 2009). In PCR amplification ST11 [5'-GCCAACCATTGCTAAATTGGCGCA-3'] and ST15 [5'-GGTAGAAATTCCCAGCGGGTACTG G-3'] genes were used as a set of primer which target *Salmonella* spp. at 429 bp (Soumet *et al.*, 1999). The reaction mixture and PCR temperature setting were modified from (Pui *et al.*, 2011).

Antibiotic Susceptibility

All the positive result from MPN-PCR were proceed with antimicrobial sensitivity test with four different antibiotic penicillin (10µg), tetracycline (30µg), trimethoprim (5µg), and ciprofloxacin (5µg) on Mueller-Hinton Agar (Difco, Labchem Sdn Bhd, Selangor). Then, all the plate was incubated for 24 hours at 37°C. The inhibition zone was measured for antimicrobial activity (NCCLS, 2003; Bauer *et al.*, 1996).

Result and Discussion

The MPN result shows that the highest estimation number of *Salmonella* sp. was found in smoked catfish to more than $24x10^7$ MPN/g while smoked meat with MPN scores of $2.9x10^7$ MPN/g. The minimum set of microbiologist limit in Malaysian and Australia Standard was 10^5 MPN/g or 10^5 cfu/g (Yusof *et al*., 2007; Food Act 1985, 2015; Australian New Zealand Food Standard Code, 2015). So, the presence of *Salmonella* sp. in Kuala Pilah stall was exceeded the safety of food microbiological limits. The highest prevalence distribution of MPN-PCR of *Salmonella* sp. was found in smoked catfish with all tubes was positive and smoked meat (50%) with four out of eight tubes was positive. The total prevalence of *Salmonella* sp. was (76%) with 13 out of 17 tubes were positive (Figure 1).

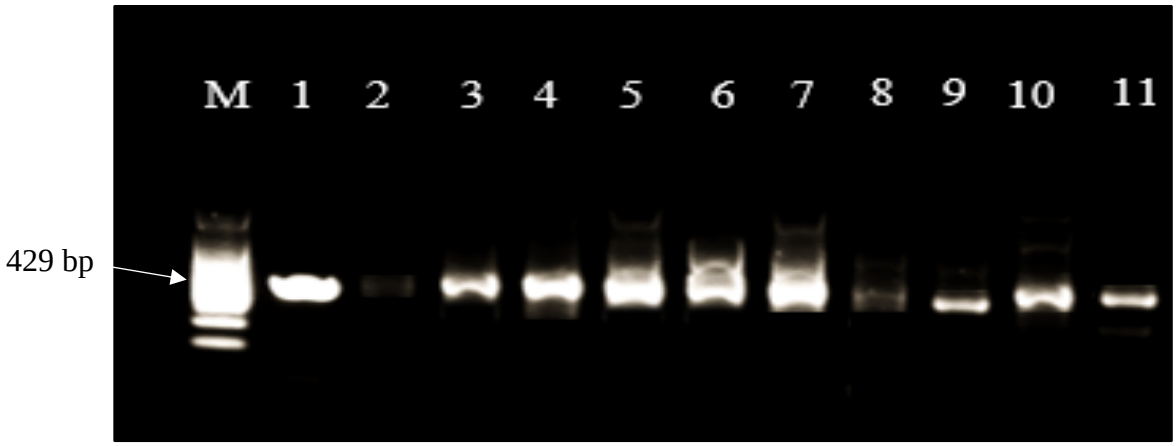

Figure 1. Representative amplification of *Salmonella* sp. (429 bp). Lane M: 100 bp DNA ladder; Lane 1: positive control at 429bp; Lane 2 to 11: representatives of positive samples.

In this study, the resistance towards penicillin was found to be 100% resistance followed by ciprofloxacin (92%), tetracycline (77%) and trimethoprim (62%). The higher level of *Salmonella* sp. resistance towards penicillin could be explained by the fact that they were the first-choice antibiotics and widely used for most disease treatment. According to Faruque (2012), the effective was to eradicate this organism was to use antibiotics that have intracellular activity since *Salmonella* sp. was an intracellular pathogens and penicillin was only inhibit cell wall synthesis. In fact, *Salmonella* sp. consist of lipopolysaccharide which make them heat stable, resistance to alcohol and dilute acids (Hu and Kopecko, 2003; Yousef and Carlstrom, 2003). As a result, the isolates show a multi-resistance patterns from one to four antibiotics tested with MAR indices ranging from 0.25 to 1.00 (Table 1). However, isolates SM17 shows the other intracellular antibiotics still could be used in treating Salmonellosis with a MAR index of 0.25.

Table 1. Antibiotics resistance patterns and multiple antibiotic resistance (MAR) index of *Salmonella* sp.

Isolates no.	Sample Location	Sample sources	Resistance Patterns	MAR index
SF1	Street Stall	Smoked Fish	PWTeCip	1.00
SF2			PCip	0.50
SF3			PTeCip	0.75
SF4			PWTeCip	1.00
SF5			PWTeCip	1.00
SF6			PCip	0.50
SF7			PWTeCip	1.00
SF8			PWTeCip	1.00
SF9			PWTeCip	1.00
SM10		Smoked Meat	PTeCip	0.75
SM12			PWTeCip	1.00
SM15			PWTeCip	1.00
SM17			P	0.25

Conclusion

In conclusion, this study had demonstrated a distribution of *Salmonella sp.* in smoked foods in Malaysia which shows that smoked catfish were found to be highly contaminated as compared to smoked meat. Therefore, smoked foods in Malaysia were exceeded the microbiologist safety limit and pose a health risk to consumers.

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