Antimicrobial resistance profile of *Salmonella* present in organic farming in Selangor, Malaysia

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**Abstract**

Presence of *Salmonella* in organic farming may lead to contamination in fresh produce. This study was designed to detect *Salmonella* contamination in organic vegetable farm situated in Serdang and to evaluate the antibiotic susceptibility profiles of the isolates. A total of 460 samples of brinjal, cucumber, ladies’ fingers and soil were collected and examined for the presence of *Salmonella*. The obtained isolates were identified and confirmed by biochemical characterization and serotyping. Antibiotic sensitivity profiles of the isolates were determined by using agar disk diffusion method. *Salmonella* spp. was detected in brinjal (1.7%, n=2), cucumber (1.7%, n=2), ladies’ fingers (2.5%, n=3) and soil (5.0%, n=5) samples. The prevalent serovars were *Salmonella enterica* ser. Senftenberg, *S. enterica* ser. Weltevreden and *S. enterica* ser. Corvallis. All strains were resistance to penicillin and vancomycin, with multiple antibiotic resistance (MAR) index between 0.21 and 0.36, demonstrated here as multi-drug resistant (MDR) *Salmonella*. The result highlighted that organic vegetables constituted potential sources of *Salmonella*, informing continuous monitoring and tightened surveillance are necessary to ensure food safety.

1. **Introduction**

Organic products especially vegetables and fruits are most consumed by all races (Malays, Chinese and Indians) in Malaysia due to free chemicals, all-natural and good for health. Despite the health benefits, the risk of microbiological contamination on fresh organic produce is concerning. Generally, fresh produce might be contaminated by foodborne pathogens which present in the soil or introduced with organic fertilizers, as well as irrigation (Kuan *et al.*, 2017). Colonization of foodborne pathogens frequently occurs during cultivation (from soil), harvesting, processing and transport. Previous studies report that raw vegetables are contaminated by foodborne pathogens like *Salmonella* spp. at the retail level, either wet markets or hypermarkets (Kuan *et al.*, 2017; Abatcha *et al.*, 2018; Saw *et al.*, 2020).

Salmonellosis is caused by ingesting *Salmonella* spp. People infected with *Salmonella* may develop diarrhoea, fever and abdominal cramps within 12 to 72 hours after
infection which could last up to seven days (Saira Banu et al., 2019; FDA, 2020). More severe cases could become fatal in which it was estimated that approximately 450 mortalities each year in the United States due to acute salmonellosis (FDA, 2020). A wide variety of antimicrobial drugs are employed to treat Salmonella infections. However, the emergence and spread of antimicrobial-resistant Salmonella isolates constitute a global challenge for the effective treatment and control of these infections (Hur et al., 2012). In particular, multidrug-resistant (MDR) Salmonella has been reported in retail vegetables (post-harvest level) either collected from organic farms or traditional farms (Abakpa et al., 2015; Wadamori et al., 2016). An earlier study found that most isolated MDR Salmonella strains such as S. enterica ser. Albany, S. enterica ser. Brancaster, S. enterica ser. Corvallis, S. enterica ser. Dusseldorf and S. enterica ser. Paratyphi B. are widely distributed in vegetables at retail level (Abatcha et al., 2018). Therefore, it is vital to strengthen or enhanced surveillance typically at the pre-harvest level, which is critical in the current era of rapidly increasing antimicrobial resistance. This is the first study to detect Salmonella spp. contamination in the organic vegetable farm (pre-harvest level) in Serdang and to evaluate antibiotic resistance patterns in the region.

2. Materials and methods

2.1 Sample collection

In this study, 120 organic vegetable samples each of brinjal, cucumber, ladies’ fingers and 100 soil samples (total 460) were collected utilizing systematic sampling method repeated for every two to three times per week from April 2018 to May 2019 at TPKM4 organic farm, Serdang (Figure 1) located near to the Universiti Putra Malaysia. All samples were brought aseptically to the laboratory for Salmonella isolation.

2.2 Isolation and identification of Salmonella

For isolation of Salmonella from vegetable and soil, 25 g and 10 g of samples were pre-enriched in 225 mL and 90 mL of buffered peptone water (BPW) (Merck, Darmstadt, Germany), respectively, followed by incubation at 37°C for 24 hrs. After pre-enrichment, 0.1 mL suspensions were added to 9.9 mL of Rappaport Vasiliadis Soy (RVS) broth (Merck, Darmstadt, Germany) and incubated at 37°C for 24 hrs. Then, the enriched samples were plated on Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24 hrs. Presumptive colonies in XLD plates were selected and subjected to biochemical characterization (decarboxylation of lysine, hydrolysis of urea, indole test) as recommended by ISO 6579:2002 (ISO, 2002). Serotyping of Salmonella isolates was carried out at Salmonella reference centre at Public Health Laboratory, Ipoh, Malaysia in accordance with the Kauffmann-White scheme.

2.3 Antibiotic susceptibility test

Antibiotic susceptibility was evaluated using disc diffusion method on the following 14 antimicrobial agents: amoxicillin (30 µg), ampicillin (10 µg),
cephazolin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg), trimethoprim (5 µg) and vancomycin (30 µg) (Oxoid, Hampshire, United Kingdom), in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2012). Multiple antibiotic resistance (MAR) index was calculated as described by Krumperman (1983).

3. Results and discussion

In this study, only 2.6% (12/460) of the samples were contaminated with Salmonella spp. For vegetable samples, Salmonella contamination in ladies’ fingers 2.5% (3/120) was greater compared to brinjal and cucumber 1.7% (2/120) (Table 1). In accordance with this finding, low prevalence rates of Salmonella spp. in vegetables have been reported in Selangor 4.0% (3/75) (Kuan et al., 2017) and Perak 3.3% (6/180) (Saw et al., 2020). The incidence of Salmonella in brinjal, cucumber and ladies’ fingers could be due to surface contamination, where the presence of epicuticular wax or uneven surfaces of the vegetable might enhance bacterial adhesion. Some vegetables are grown on the ground which may allow Salmonella present in the soil to cause the contamination. In the present study, we observed 5.0% (5/100) Salmonella in soil samples, which add further support to this vegetable contamination. Perhaps other factors, such as environmental and handling conditions, that would impact the microbial profile of these vegetables.

Out of 12 isolates, 5 serotyped as S. enterica ser. Senftenberg indicating a prevalence rate of 41.67%. Other isolates identified as S. enterica ser. Weltevreden (n=4), and S. Corvallis (n=3). Similarly, the presence of 16.09% S. enterica ser. Weltevreden (n=14) and 10.34% S. Corvallis (n=9) were also found at retail fresh markets in different states of Malaysia including Selangor (Abatcha et al., 2018). On the contrary, a lower (10.1%) rate of S. enterica ser. Senftenberg incidence has been reported in raw vegetables in the same region (Salleh et al., 2003). Meanwhile, the absence of S. enterica ser. Enteritidis and S. enterica ser. Typhimurium in the current study is of interest, as both are well-known serovars to have great concern as foodborne salmonellosis worldwide (Hur et al., 2012). However, a few studies have detected S. enterica ser. Enteritidis in Malaysia (Kuan et al., 2017; Abatcha et al., 2018), and S. enterica ser. Typhimurium in Brazil (Sant’Ana et al., 2011), Nigeria (Abakpa et al., 2015) and New Zealand (Wadamori et al., 2016) from different retail vegetables.

We evaluated the resistance profile of Salmonella strains against 14 antimicrobial agents. As shown in Table 2, two antibiotics gentamicin and trimethoprim were found to be 100% effective. However, a high prevalence of resistance was observed for erythromycin (75.0%), streptomycin (41.7%), cephalxin (33.3%), tetracycline (33.3%) and ampicillin (25.0%). Moreover, all isolates were resistant (100%) to penicillin and vancomycin. Similar resistance to antibiotics has been reported for Salmonella spp. from vegetable and soil samples to penicillin and vancomycin (Wadamori et al., 2016). Previous studies also reported a high level of resistance among Salmonella isolates including S. enterica ser. Corvallis and S. enterica ser. Weltevreden from vegetables to streptomycin (66.6%), tetracycline (44.3%) and ampicillin (26.7%) (Abatcha et al., 2018).

Based on the antibiotic resistance profile (Table 3), all isolates were found to be resistant to at least three different classes of antibiotics. The highest MAR index (0.36) was recorded for ladies’ fingers and soil isolates, which found in S. Weltevreden with a resistance pattern of KZ-E-P-Te-V. All S. Senftenberg and S. Corvallis isolates were resistant to four and three antibiotics with MAR index of 0.29 and 0.21, respectively. These results suggest that MDR (resistant to three or more antimicrobial agents) Salmonella isolates are prevalent in organic farming.

According to certified organic farming practice in Malaysia, there is no use of agricultural chemicals or antimicrobials to produce fresh vegetables. However, other variables, such as the use of contaminated

Table 1. Prevalence of Salmonella in brinjal, cucumber, ladies’ fingers and soil

<table>
<thead>
<tr>
<th>No.</th>
<th>Source</th>
<th>No. of samples</th>
<th>No. of positive samples</th>
<th>Percentage of positive sample</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Brinjal</td>
<td>120</td>
<td>2</td>
<td>1.7%</td>
<td>S. enterica ser. Senftenberg (n=2)</td>
</tr>
<tr>
<td>2</td>
<td>Cucumber</td>
<td>120</td>
<td>2</td>
<td>1.7%</td>
<td>S. enterica ser. Senftenberg (n=1) and S. enterica ser. Corvallis (n=1)</td>
</tr>
<tr>
<td>3</td>
<td>Ladies’ fingers</td>
<td>120</td>
<td>3</td>
<td>2.5%</td>
<td>S. enterica ser. Corvallis (n=1) and S. enterica ser. Weltevreden (n=2)</td>
</tr>
<tr>
<td>4</td>
<td>Soil</td>
<td>100</td>
<td>5</td>
<td>5.0%</td>
<td>S. enterica ser. Senftenberg (n=2) and S. enterica ser. Weltevreden (n=2)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>460</td>
<td>12</td>
<td>2.6%</td>
<td></td>
</tr>
</tbody>
</table>
irrigation water and incorrect composting process may explain the occurrence of MDR isolates in this study. Indeed, high levels of enteric bacteria, protozoa or helminths are present in contaminated irrigation water, resulting in a high frequency of pathogen detections from harvested fresh produce (Abakpa et al., 2015; Saw et al., 2020). Furthermore, the use of manure from animal faeces, especially from the source of poultry houses, may have significantly contributed to the emergence of MDR strains of Salmonella faeces, especially from the source of poultry houses, may have significantly contributed to the emergence of MDR isolates in this study. Therefore, strict surveillance and sustainable agriculture practices at the pre-harvest stage are needed to ensure food safety.

4. Conclusion

Our results highlight the potential of organic vegetables may act as reservoirs in harbouring MDR Salmonella. The data provide opportunities for management of antibiotic-resistant Salmonella and risk assessment in the region.

Conflict of interest

The authors declare no conflict of interest.
disk Susceptibility Tests. Wayne, PA: CLSI.


