Contamination of antibiotic-resistant bacterial pathogens in raw food is becoming an increased health risk in numerous countries, including Vietnam where raw herbs and vegetables are used daily in many dishes as a flavor enhancer and a source of vitamin and nutrients. However, raw vegetables can also be a reservoir of various foodborne pathogens such as Pseudomonas spp. and Enterobacteriaceae. In this study, we evaluated the extent of Pseudomonas spp. contamination in 180 ready-to-eat (RTE) vegetables samples from restaurants in Hanoi, examined their antibiotic susceptibility profiles and determined the ability to produce β-lactamase enzymes of Pseudomonas spp. strains. Our results showed that 21.67% (n = 39) of ready-to-eat vegetables samples in Hanoi were contaminated with Pseudomonas spp.. Of those, sixteen samples were determined to be β-lactamase producing strains including Pseudomonas putida, P. mendocina and P. aeruginosa. Further analysis revealed six strains (37.50%) producing extended spectrum β-lactamase (ESBL) enzyme, five strains (31.25 %) producing ampC β-lactamase enzyme and five strain (31.25 %) producing both ESBL and ampC β-lactamases. It can be concluded that ready-to-eat vegetables in Hanoi would be a source of contamination of β-lactamase producing Pseudomonas spp. that could pose a threat to public health in the community.

**Keywords:** food, ready-to-eat vegetables, contamination of antibiotic-resistant bacterial pathogens, Pseudomonas spp., health risks.

Ready-to-eat raw vegetables have become a favorable product among many consumers thanks to their high vitamin contents and low energy. In Vietnam, vegetables are common food in the daily life. According to the World Bank’s report (2017), the consumption rate of vegetables in Vietnam is 0.4 kg/day/person, therefore, it can be estimated that 2,800 tons of vegetables are consumed everyday in Hanoi [1]. With a consumption of about 1 million tons per year, Hanoi must use supplies from vicinity provinces in the Red River Delta. However, vegetables can become a source of bacterial contamination to animals and humans due to polluted irrigation water and improper washing before they are sold at a marketplace. Thus, it is difficult to control hygiene and monitor safety for food product.

*Pseudomonas spp.* are versatile gram-negative bacteria that occur in various habitats such as soil, water and living organisms including animals, insects and human. In the genus, *P. aeruginosa* are a risk to human health as they cause serious diseases such as septicemia, liver damage or necrosis at in-
jured sections. Other *Pseudomonas* species including *P. fluorescens, P. luteola, P. putida*, and *P. stutzeri* possess lower toxicity, but they can cause many infectious diseases in immune-compromised patients [2, 3]. *Pseudomonas* spp. often invade host tissue and cause infection and septicemia in immune-compromised hosts such as patients suffering from HIV/AIDS, cystic fibrosis, bronchiectasis and severe chronic obstructive pulmonary, burn, or patients after malignant or urinary removal [4]. Recently, pathogenic *Pseudomonas* spp. have become one of the prominent public health concerns due to their fast-evolving multi-drug resistance. Studies on disease treatment in patients show difficulties in combatting high multi-drug resistant *Pseudomonas* spp. [5].

Studies on bacteria extracted from raw vegetables sold at different markets and restaurants that have antibiotic resistant agents have been previously reported [5]. The prominent group of antibiotic resistant bacteria contaminants from raw vegetables is *Pseudomonas* spp. They are well known to have multiple antibiotic resistances due to gene scarring genetic factors that originated from other Gram-negative bacilli families such as *Enterobacteriaceae* through gene exchange [6]. Among these, the β-lactamase producing strains especially extended-spectrum β-lactamase (ESBL) and plasmid-encoded β-lactamase (AmpC) producers are of greatest interest. The emergence and spread of β-lactamase have become a significant global health problem, as they inactivate a wide group of antibiotics used in various hospital treatments [3]. However, until now, there have been no data on *Pseudomonas* spp. and β-lactamase producing *Pseudomonas* spp. in ready-to-eat vegetables in Vietnam. In this study, we aim to evaluate the presence of β-lactamase producing *Pseudomonas* spp. in ready-to-eat raw vegetables from restaurants in Hanoi.

**Materials and methods.** **Vegetables sampling.** A total of 180 ready-to-eat raw vegetables samples were collected at restaurants in six urban districts of Hanoi from July to October, 2018. Six districts including Cau Giay, Hoang Mai, Ha Dong, Dong Da, Hai Ba Trung, Long Bien were selected based on the high rate of population. After being acquired from restaurants, vegetables samples were finely-sliced without any further washing but brown-leaf removing. For every sample, the amount of 25 grams of sample was added into 225 mL of buffered peptone water (BD, America). The mixture was then homogenized for 30 seconds using a homogenizer. Weight 25 grams of each finely-sliced leaf-sample and homogenizing for 30 seconds using homogenizer. The mixtures were then diluted to desired concentration by using peptone (0.1 %) saline diluent.

**Bacterial isolation and identification.** *Pseudomonas* spp. are extracted with a conventional method according to ISO 13720: 2010 [7]. Briefly, 0.1 mL of homogenized sample was spread on *Pseudomonas CFC/CN* (Merck, Germany) agar supplemented with CFC Selective supplement (Merck, Germany) and the plates were incubated under aerobic conditions at 25 °C for 44 ± 4 hours. After incubation, colonies were confirmed using Oxidase Strips (Merck, Germany). Oxidase-positive colonies identified as *Pseudomonas*. *E. coli* ATCC25922 (ATCC, America) were used as negative control while *P. aeruginosa* ATCC 27853 (ATCC, America) strain was used as the positive control. Confirmed *Pseudomonas* spp. strains were further analyzed using Vitek®MS system (BioMerieux, France) to identify their species. Vitek®MS procedure was performed according to manufacturer’s recommendations.

**Identification of β-lactamase producing *Pseudomonas* spp.** To determine *Pseudomonas* spp. producing β-lactamase, susceptibility testing with disc diffusion method was performed and interpreted following the technique recommended by the World Health Organization and the guidelines by
the American Institute for Testing and Clinical Standards (CLSI) [8, 9]. The antibiotics being examined included cefotaxime (CTX, Liofilchem, Italy), cefotaxime combined with clavulanic acid (CTL, Liofilchem, Italy), ceftazidime (CAZ, Liofilchem, Italy) and ceftazidime combined with clavulanic acid (CAL, Liofilchem, Italy). The strains are confirmed to be extended-spectrum β-lactamase (ESBL) producing ones when the observed CTL or/and CAL resistance zones are at least 5 mm greater than the observed CTX and CAZ resistance zones.

The presence of AmpC β-lactamase enzyme was determined by testing strains against cefotaxime (CTX, Liofilchem, Italy), cefotaxime combined with cloxacillin (CTC, Liofilchem, Italy), ceftazidime (CAZ, Liofilchem, Italy) and ceftazidime combined with cloxacillin (CAC, Liofilchem, Italy) following CLSI guidelines. When the observed CTC or/and CAC resistance zones are at least 5 mm greater than the observed CTX and CAZ resistance zones, the tested bacteria are confirmed to be AmpC β-lactamase producing strains.

**Results and discussions.** *Pseudomonas spp. in raw vegetables.* The obtained results revealed that 39 over 180 samples of ready-to-eat vegetables collected at restaurants (Grilled duck, bun cha, bread, spring rolls, pho, vermicelli, and noodle) were found to be positive with *Pseudomonas spp.* assuming oxidase-positive colonies. The finding of this study revealed that *Pseudomonas spp.* were prevalent (Figure 1). The difference in *Pseudomonas spp.* prevalence among sampling locations (districts) was not significant (P > 0.05).

Similar to our results, the prevalence of *Pseudomonas spp.* on ready-to-eat vegetable was also reported in former studies in several countries such as Italy (n = 24), India (n = 12 isolates were collected from tomato, cucumber and potato), and Nigeria (n = 82) [5, 10, 11]. *Pseudomonas spp.* are known to be the most common bacteria involved in spoilage of many kinds of foods, due to their very simple nutritional requirements and metabolic versatility that allows them to live in variable environments. The soil and irrigation water contamination may cause the prevalence of *Pseudomonas spp.* in ready-to-eat vegetable. Numerous studies have demonstrated that water plays important role in directly introducing bacteria into food during pre-harvest and postharvest stages [12–14]. It is during the pre-harvest phase that vegetable might be contaminated with *Pseudomonas spp.* due to infected irrigation water. Furthermore, for postharvest processes, ranging from storage and washing before selling, might lead to contamination.

Identified *Pseudomonas spp.* by using *Vitek®MS.* The obtained colonies were identified on the *Vitek®MS.* We detected 3 strains of *Pseudomonas spp.* including opportunistic
pathogens (Pseudomonas aeruginosa, Pseudomonas mendocina, and Pseudomonas putida). Among them, P. putida are accounted for the majority with 36 out of 39 strains (92%). There were also 2 strains of P. aeruginosa (5%) and 1 strain of P. mendocina (3%) (Figure 2).

The identification results show a similarity of more than 90% compared to the source library of the VITEK MS identifier system, from 99.4% to 99.9% for P. putida and 99.9% for P. aeruginosa and P. mendocina (Figure 3). The results in this study are similar to those obtained in the previous research on Pseudomonas spp. presence in raw vegetables by Caldera et. al. (2016), and the study by Franzetti et. al. performed on vegetable samples collected in Milan in 2007 as well [10, 15]. Another study conducted by Devarajan et. al. (2017) in Congo, India and Switzerland revealed occurrence of 141 strains of Pseudomonas spp. in untreated hospital wastewater samples and domestic wastewater sources; P. putida (42%) and P. aeruginosa (39%) were the majority in detected Pseudomonas spp. Another study by Kittinger et. al. conducted in 2016 on water of the Danube river (Austria) showed that 66.0% (n = 520) isolates were identified as Pseudomonas putida and 27.1% were Pseudomonas fluorescens, 2 Pseudomonas aeruginosas strains, and less than five other Pseudomonas strains. A large number of Pseudomonas spp. was also found in raw and fermented vegetables in previous studies [16, 17]. The presence of animal dung containing Pseudomonas spp. used as a fertilizer or accidentally in the soil can be a source of contamination of Pseudomonas spp. on vegetables. Moreover, in most developing countries as well as Vietnam, wastewater is discharged from sewage directly into rivers and lakes that are used as irrigation water sources for cultivation, leading to Pseudomonas spp. occurrence in raw vegetable products. Meanwhile, should improper cleaning steps be applied at restaurants they are not effective enough and don’t allow removing bacteria from food products making ready-to-eat vegetables infected.

![Figure 2. Percentage of Pseudomonas spp. isolates in this study](image)

![Figure 3. Pseudomonas aeruginosa spectrum of isolate from ready-to-eat vegetable](image)
Table 1

| Pseudomonas spp. | District          | Vegetables                                      | ESBL | AmpC | ESBL &ampC |
|------------------|-------------------|-------------------------------------------------|------|------|-------------|
| P. aeruginosa    | CauGiay           | Saw-leaf, Perilla leaf, Vietnamese basil         | +    | +    | +           |
| P. putida        | Thai basil        | +                                                | -    | -    | -           |
| P. putida        | Buttercrunch lettuce, Vietnamese basil, Mint leaf | +    | +    | +           |
| P. putida        | Perilla leaf, mint leaf | -                                            | +    | -    | -           |
| P. putida        | Vietnamese Balm, Perilla leaf, mint leaf, cucumber | +    | -    | -           |
| P. putida        | Cilantro, Vietnamese Balm, Buttercrunch lettuce | -    | +    | -           |
| P. putida        | Mint leaf, Buttercrunch lettuce                  | +    | -    | -           |
| P. putida        | Vietnamese Balm, Buttercrunch lettuce            | -    | +    | -           |
| P. putida        | Mint leaf, Lettuce, Vietnamese Balm              | +    | -    | -           |
| P. putida        | Buttercrunch lettuce, Perilla leaf, Cilantro     | -    | +    | -           |
| P. putida        | Green Onion, Mint leaf                            | +    | +    | +           |
| P. putida        | Thai basil, Saw-leaf                               | +    | +    | +           |
| P. aeruginosa    | Hai Ba Trung     | Thai basil, Saw-leaf                                | +    | +    | +           |
| P. putida        | Cilantro, Green onion                              | +    | -    | -           |
| P. putida        | Buttercrunch lettuce                               | -    | +    | -           |
| P. putida        | Buttercrunch lettuce, Perilla leaf                | +    | -    | -           |

Note: + Positive  
- Negative

3.3 *Pseudomonas* spp. producing β-lactamase enzyme. 39 identified strains of *Pseudomonas* spp. were used for antimicrobial testing corresponding to each β-lactamase enzyme. The results showed that 16 strains of *Pseudomonas* spp., which were detected in vegetables from restaurants in 6 districts, produced β-lactamase enzyme, of which 6 strains produced an expanded spectrum of β-lactamase enzyme (ESBL), 5 *Pseudomonas* strains produced enzyme β-lactamase AmpC, and 5 strains produced enzyme ESBL and AmpC as well. Whole 16 strains, were *P. putida* and *P. aeruginosa*, in which 2 *P. aeruginosa* and 3 *Pseudomonas putida* produced both ESBL and β-lactamase enzyme (Table 1).

In the study conducted in Nigeria in 2016 by Odumosu et. al. *P. aeruginosa* were detected in 54 out of 82 vegetable samples; 10 out of these 54 strains were identified with ESBL enzymes [5]. The result of Odumosu’s study is higher than ours, due to the difference in geography, sample size, conditions of cultivation and sanitation as well. Despite of the fact that ready-to-eat vegetables are a good source of vitamins and nutrients, they can also be a source of pathogenic contamination hazardous for humans and transfer antibiotic-resistant bacteria to them.

**Conclusion.** The study showed prevalence of 16 *Pseudomonas* spp. strains producing β-lactamase enzyme in 180 samples of ready-to-eat vegetables collected in Hanoi. The number of *Pseudomonas* member found in ready-to-eat vegetable in our study was lower than in former studies from some countries worldwide. The difference in geography, sample size and sample collection location might lead to different results among studies.

It is necessary to conduct further studies on genetic characteristics and ability to transfer antibiotic resistant genes to other gram-negative bacteria in same ecological conditions.

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**Conflict of interests.** The authors declare there is no any conflict of interests.
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