Proceeding Paper

Prevalence and Antibiogram Profiling of Extended-Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* in Raw Vegetables, in Malaysia †

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Abstract: The prevalence of antimicrobial resistance has drawn the public's attention worldwide. The presence of ESBL *E. coli* in fresh produce and other food represents a growing problem involving food safety and has become a global food safety issue. This study was aimed to determine the prevalence of ESBL-producing *E. coli* in raw vegetables (lettuce and bean sprouts) from hypermarkets and wet markets and to establish the antibiogram of the isolates. In this study, a total of 180 samples (95 samples of lettuce and 85 samples of bean sprouts) were collected from hypermarkets and wet markets. The most-probable-number analysis and multiplex polymerase chain reaction (MPN–PCR) was used to detect and quantify the ESBL-producing *E. coli* in raw vegetable samples. The prevalence rates of ESBL-producing *E. coli* in lettuce and bean sprouts were 62.11% (59/95) and 63.53% (54/85), respectively, with a microbial load range of <3 to >1100 MPN/g. A total of 15 isolates of ESBL-producing *E. coli* recovered from the samples were tested with an antibiotic susceptibility test (AST) with different antibiotic classes. All isolates were found to be susceptible to cefepime, piperacillin/tazobactam, and meropenem. A total of nine ESBL-producing *E. coli* strains showed multidrug resistance. In conclusion, the high prevalence rate of ESBL-producing *E. coli* in raw vegetables showed that raw vegetables could act as a potential vehicle to transmit ESBL-producing *E. coli* to the human population.

Keywords: raw vegetables; ESBL-producing *E. coli*; most probable number–polymerase chain reaction (MPN–PCR); antibiogram

1. Introduction

The prevalence of extended-spectrum beta-lactamase (ESBL) bacteria in food chain has become a global food safety issue. The infections caused by ESBL-producing *E. coli* include intra-abdominal abscesses, peritonitis, urinary tract infection (UTI), and in more severe cases may lead to blood poisoning. Nowadays, vegetables are served as convenient meals, and they have begun to take the center-stage of main meals. However, raw vegetables were identified as the commodity group of the greatest concern from a microbiology safety perspective [1]. This study was aimed to determine the prevalence rate of ESBL-producing *E. coli* in raw vegetables and the antibiogram of the ESBL-producing *E. coli* isolates.

2. Materials and Methods

2.1. Sample Collection

A total of 180 samples of raw vegetables (lettuces, $n = 95$; bean sprout, $n = 85$) from the wet market and hypermarket were purchased randomly in the Serdang area, Malaysia. All the samples were placed into a sterile plastics bag and analysed immediately upon arrival at the lab.

2.2. Most Probable Number and Polymerase Chain Reaction (MPN-PCR)

A 10 g sample was placed in a sterile stomacher bag with 90 mL of Tryptic Soy Broth and homogenized for 1 min. The suspensions were then diluted 10-fold serially to 1000-fold. The three-tube MPN method was carried out by transferring each dilution (1 mL) into triplicate MPN tubes containing 10 mL of MacConkey broth. All the tubes were incubated at 37 °C 24 h. After incubation, the positive tubes were examined before genomic DNA extraction. Four sets of primers were used to detect the presence of ESBL-producing *E. coli* including 16S rRNA gene of *E. coli*, bla_{TEM}, bla_{SHV}, and bla_{CTX-M}.

2.3. Antibiotic Susceptibility Test (AST)

A total of 15 ESBL-producing *E. coli* strains were isolated from raw vegetables by using Chromogenic Brilliance ESBL agar and further confirmed by PCR analysis. All the isolates were tested against different antibiotics including piperacillin/tazobactam (TZP, 110 µg), meropenem (MEM, 10 µg), aztreonam (ATM, 30 µg), ciprofloxacin (CIP, 5 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), ceftriaxone (CRO, 30 µg), cefepime (FEP, 30 µg), ampicillin (AMP, 10 µg), amoxicillin/clavulanic acid (AMC, 30 µg). The susceptibility range of the selected antibiotic was based on CLSI (2017) [2]. The diameter of the inhibition zone was measured and recorded to determine the susceptibility level of each antibiotic. Isolates with resistance to more than three classes of antibiotics are considered multidrug-resistant bacteria.

3. Results and Discussion

Of the 180 vegetable samples (lettuce, $n = 95$; bean sprouts, $n = 85$) tested, 113 (63.12%) yielded ESBL-producing *E. coli*. The contamination rates were 62.11% (59/95) in lettuce and 63.53% (54/85) in sprouts. Based on Figure 1, the prevalence of rate ESBL producing *E. coli* in lettuce and bean sprouts from wet market is higher compare to hypermarket. The ESBL-producing *E. coli* detected in vegetable samples from the wet market (73.86%) were significantly ($p < 0.05$) higher than the vegetable samples from the hypermarket (52.74%). The contamination of raw vegetables can happen via different pathways including pre-harvest (fertilizer, irrigation water and soil) and post-harvest process (improper handling and storage) [3]. The different prevalence rates of ESBL-producing *E. coli* in raw vegetables from hypermarket and wet market may be due to the display and storage methods, as well as improper handling by the food handlers.
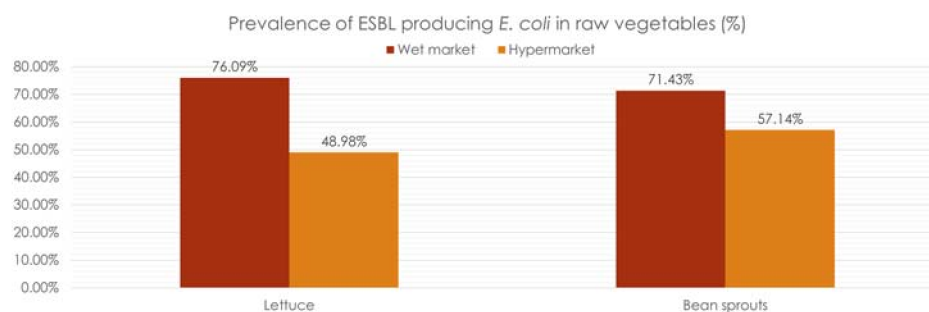

Figure 1. Prevalence rate of ESBL-producing *E. coli* in raw vegetables from wet markets and hypermarkets.

In AST, a total of nine isolates (60%) showed multidrug resistance. The antibiotic resistance pattern of ESBL-producing *E. coli* strains were showed in Figure 2.
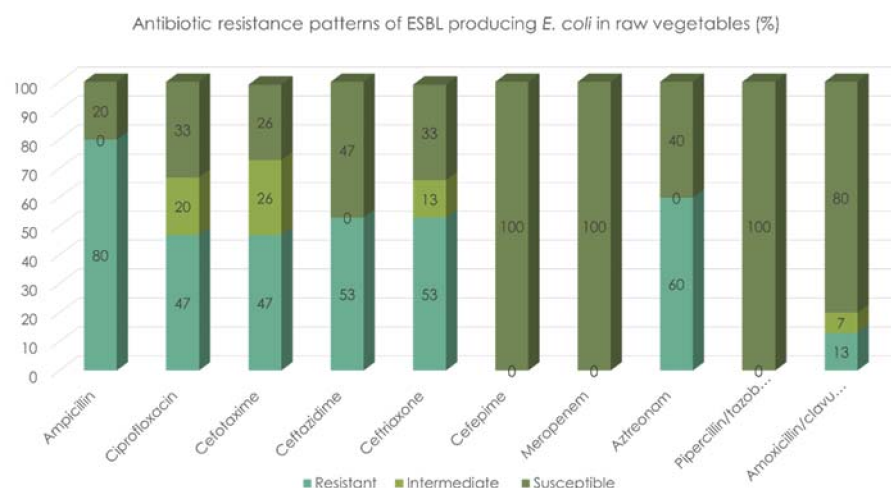
Figure 2. The antibiotic resistance patterns of ESBL-producing *E. coli* in raw vegetables.

Ampicillin is known as one of the regular traditional antibiotic treatments; however, 80% of the ESBL-producing *E. coli* strains in this study showed resistance to ampicillin. The antibiotic resistance patterns may vary across geographical location as the guidelines of antibiotics usage vary from country to country [4,5].

4. Conclusions

The high prevalence rate of ESBL-producing *E. coli* in raw vegetables indicated that raw vegetables may act as a potential vehicle to transmit ESBL-producing *E. coli* and ESBL genes to humans. The prevalence rate of ESBL-producing *E. coli* in lettuce from wet markets was significantly higher than the lettuce in hypermarket. However, there is no significant difference between the contamination rate of ESBL-producing *E. coli* in bean sprouts from hypermarkets and wet markets. The antibiotic resistance pattern of isolated ESBL-producing *E. coli* showed 60% of the ESBL-producing *E. coli* are multidrug resistant.

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