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

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Abstract: The widespread 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 179 samples (95 samples of lettuce and 84 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 rate of ESBL producing *E. coli* in lettuce and bean sprouts were 62.11% (59/95) and 64.29% (54/84), 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 antibiotic susceptibility test (AST) with different antibiotic classes. All isolates were found 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

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1. Introduction

The widespread of extended-spectrum beta-lactamase bacteria in food chain have become a global food safety issue. The infections caused by ESBL producing *E. coli* included intra-abdominal abscesses, peritonitis, urinary tract infection (UTI) and more severe may lead to blood poisoning. Nowadays, vegetables served as convenient meals and has become a trend to take over 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 179 samples of raw vegetables (lettuces, n = 95; bean sprout, n = 84) from the wet market and hypermarket were purchased randomly in the Serdang area, Malaysia. All the samples were collected 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 of the 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. 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 set 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 antibiotic 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). The diameter of the inhibition zone was measured and recorded to determine the susceptibility level of each antibiotic. The isolate that resistance more than three class antibiotics is considered as 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. The ESBL producing *E. coli* detected in vegetable samples from wet market (73.86%) were significantly ($p < 0.05$) higher than the vegetable samples from 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 rate of ESBL producing *E. coli* in raw vegetables from hypermarket and wet market may due to the displayed and storage method, and improper handling by the food handlers.

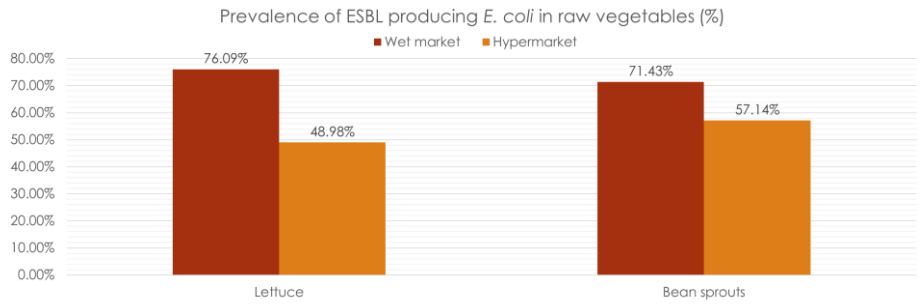

Figure 1. Prevalence rate of ESBL producing *E. coli* in raw vegetables from wet markets and hypermarkets.

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.

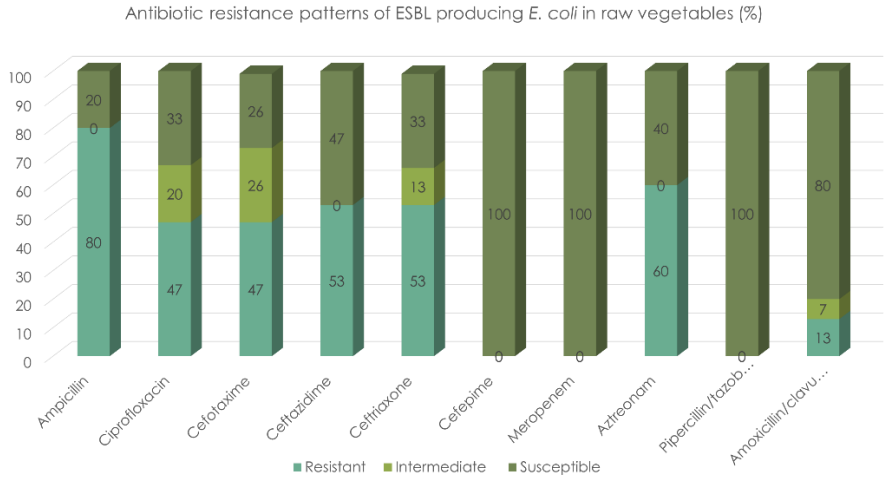

Figure 2. The antibiotic resistance patterns of ESBL producing *E. coli* in raw vegetables.

Ampicillin is known as one of the regular traditional antibiotic treatments, however, 80% of the ESBL producing *E. coli* strains in this study showed resistant to ampicillin. The antibiotic resistance patterns may vary across geographical location due to the guidelines of antibiotics usage are varies 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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