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Detection and antibiotic resistance profile of extended-spectrum beta-lactamase—producing *Escherichia coli* in raw vegetables

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Abstract

The increase of antibiotic-resistance bacteria in food and vegetables has heightened the concern related to food safety globally. The current study aimed to determine the prevalence and antibiotic resistance profile of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in raw vegetables. This study coupled the most probable number (MPN) method with multiplex polymerase chain reaction (mPCR) to determine the presence and enumerate the ESBL-producing *E. coli* in raw vegetables. The result showed that the prevalence of the isolates in raw vegetables was 62.78% (113/180), consisting of 62.11% (59/95) in green carol lettuce and 63.53% (54/85) in mung bean sprouts, whereas the microbial load ranged from <3 to >1100 MPN/g with median 9.2 MPN/g. Overall, the study showed that there was no significant difference (P>0.05) in the microbial density of the isolates in vegetable samples purchased from wet markets and hypermarkets. Furthermore, the antibiotic susceptibility test revealed that all related strains were susceptible to cefepime, piperacillin/tazobactam and meropenem. However, the resistance to ampicillin was shown by 80% of the isolates. The multiple antibiotic resistance (MAR) indices of ESBL-producing *E. coli* ranged from 0.1 to 0.6. The majority of the isolates (60%) showed multidrug resistance. Hence, the current study suggested that raw vegetables could be a vehicle for the transmission of ESBL-producing *E. coli* to humans.

1. Introduction

Vegetables provide essential nutrients and fibre that help to maintain human health. Over the past few years, raw veganism has become a trend. The proponents of this diet only consume raw or minimally processed fresh produce and claim that the cooking process will affect the vegetables' nutritional value (Rickman *et al*., 2007). However, consuming raw vegetables might cause the ingestion of foodborne bacteria and thus pose a safety threat (Reuland *et al*., 2014; van Hoek *et al*., 2015; Waturangi *et al*., 2019). Vegetables can be contaminated by water sources, soil, production inputs like manure, or human handling in the production steps (Macieira *et al*., 2021). In 2011, an outbreak of Vero-toxigenic *E. coli* (VTEC) O104 connected to Shiga-toxin and ESBLs was reported in Germany and the consumption of raw vegetables was associated with the outbreak (Buchholz *et al*., 2011). *Enterobacteriaceae*, mainly *Klebsiella pneumoniae* and *E. coli* produce extended-spectrum beta -lactamases (ESBLs) as a mechanism to render antibiotics ineffective, thus causing severe damage to the infected individuals. The infections caused by these bacteria lead to excess mortality, morbidity and treatment costs (Friedman *et al*., 2016; Aliyu *et al*., 2016). Extended-spectrum beta-lactamase was defined as the enzymes produced by *Enterobacteriaceae* that confer resistance to the majority of beta-lactam antibiotics, which include penicillin, monobactam (aztreonam), and broad-spectrum cephalosporins, except cephamycin and carbapenems (Paterson and Bonomo, 2005; Shaikh *et al*.,

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2015; Saisi *et al*., 2019). According to Paterson and Bonomo (2005), this enzymatic activity can be inhibited by beta-lactamase inhibitors. The inhibitors are co-administered with the antibiotics to make them more effective in killing the bacteria.

The rise of antibiotic-resistant microorganisms has received global attention not only due to their impact on population health but also reflects food safety issues and environmental integrity. According to the antibiotic-resistant (AR) threats report (CDC, 2019) in the United States, more than 2.8 million cases of infections in the country are associated with antibiotic resistance every year which leads to at least 35,000 deaths. The Ministry of Health (MOH) Malaysia (2017) also claimed that the infections caused by extended-spectrum beta-lactamase (ESBL)- producing *E. coli* marked up from 15% in 2010 to 23.4% in 2016 (Antibiotic Resistance Surveillance Reference Laboratory, Institute for Medical Research, Malaysia, 2017). The overuse and misuse of antibiotics in the healthcare system, livestock industry, and farming practices are the major contributors to this phenomenon (Durso and Cook, 2014; Ben Said *et al*., 2015; Yaici *et al*., 2017).

Recently, research has revealed the occurrence of ESBL-producing bacteria in clinical samples (Lim *et al*., 2009; Hashim *et al*., 2011; Ho *et al*., 2012), livestock and related products (Brower *et al*., 2015; Aliyu *et al*., 2016; Falgenhauer *et al*., 2019; Song *et al*., 2020). However, reports on the prevalence of ESBL-producing bacteria in raw vegetables are limited. Hence, the present study aimed to determine the prevalence of ESBL-producing *E. coli* in raw vegetables from different markets using the MPN-PCR method and to identify the antibiotic resistant profile of the isolates. To the best of our knowledge, this study is the first study conducted to determine the prevalence of ESBL-producing *E. coli* in raw vegetables in Malaysia.

2. Materials and methods

2.1 Sample collection

A total of 180 raw vegetable samples comprising 95 green carol lettuce (*Lactuca sativa* var *crispa*) and 85 mung bean sprouts (*Vigna radiata*) were randomly collected from different local wet markets and hypermarkets. The sample was placed in a sealed sterile bag individually and carried in an ice box to the laboratory for subsequent analysis.

2.2 Sample processing and most-probable-number enrichment

This study employed the three-tube most probable number (MPN) method. A total of 10 g of vegetable samples was homogenized with 90 mL of tryptic soy broth (TSB) in a sterile stomacher bag for 1 min. Serial dilution was carried out up to the 10^{-4} dilution. For each dilution, 1 mL of the diluent was transferred to 9 mL MacConkey broth. The tubes were then incubated at 37° C for 24 hrs. All samples were analysed in triplicates. Positive MPN tubes were identified via the change of the solution colour from purple to yellow with gassing. The positive MPN tubes were then subjected to isolation of ESBL-producing *E. coli* and genomic DNA extraction for PCR analysis.

2.3 Isolation of extended-spectrum beta-lactamase-producing Escherichia coli

The isolation of ESBL-producing *E. coli* was performed by streaking an inoculum from the positive MPN tubes onto Eosin Methylene Blue (EMB) agar plates. The agar plates were incubated at 37°C for 18-24 h. Presumptive colonies with green metallic sheen on EMB agar were identified as *E. coli*. The presumptive colonies were further confirmed by subculturing on MacConkey agar and Chromogenic Brilliance ESBL agar. Colonies that were dark pink on MacConkey agar were confirmed as *E. coli* while blue or pink colonies on Chromogenic Brilliance ESBL agar were confirmed as ESBL-producing *E. coli*.

2.4 Genomic DNA extraction and multiplex-PCR amplification

The positive MPN tubes were subjected to DNA extraction using the boiled-cell method as described by Kwan *et al*. (2019). To collect the bacterial pallet, an aliquot from the positive MPN tubes was centrifuged at 12,000×*g* for 2 mins. After centrifugation, the pellet was added to 500 µL sterile distilled water for re-suspension and boiled for 10 min using a dry bath. The suspension was then cooled at -20°C for 10 mins. Finally, the mixture was centrifuged at 12,000×*g* for another 2 mins and the supernatant was used as a DNA template for multiplex PCR.

The multiplex PCR was performed to detect the presence of the 16S rRNA gene of *E. coli* and the ESBL genes (bla_{SHV}, bla_{TEM} and $bla_{\text{CTX-M}}$). Table 1 shows the sequences of the primers used for multiplex PCR. A total of 25 µL reaction mixture, which contained 5 µL PCR buffer (5×), 3 µL $MgCl_2$ (25 mM), 0.5 µL deoxynucleotide triphosphate (10 mM), 0.5 µL of each primer (0.5 mM), 0.2 µL of *Taq* polymerase (5U/µL) and 3 µL of DNA template was prepared for the amplification. The mPCR was performed according to the following condition: initial denaturation at 94°C for 5 mins, 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 30 s, extension at 72°C for 1 min,

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Table 1. Primers used in the multiplex polymerase chain reaction.

Target gene	Primer sequence (5'-3')	Size (bp)	Reference
16S rRNA of *E. coli*	(F) GGAAGAAGCTTGCTTCTTGCTGAC (R)GCCCGGGGATTTCACATCTGACTTA	544	Sabat *et al*. (2000)
bla_{SHV}	(F) TCAGCGAAAAACACCTTG (R) CCCGCAGATAAATCACCA	471	Zaniani *et al*. (2012)
bla_{TEM}	(F) GAGTATTCAACATTTCCGTGTC (R) TAATCAGTGAGGCACCTATCTC	861	Zaniani *et al*. (2012)
bla_{CTX-M}	(F) TACCGCAGATAATACGCAGGTG (R) CAGCGTAGGTTCAGTGCGATCC	355	Chia *et al*. (2005)

and final extension at 72°C for 7 mins. The PCR products were electrophoresed on 1.2% (wt/vol) agarose gel stained with ethidium bromide at 70 V for 45 mins. The gel was then visualized under UV light using the gel documentation system.

2.5 Antibiotic susceptibility test and multiple antibiotics resistance index

The antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method described in the Clinical and Laboratory Standard Institute (CLSI) (2017). The 0.5 McFarland bacterial suspension was prepared by suspending one to two bacterial colonies of confirmed isolates in a saline solution. The inoculum was swabbed with a sterile cotton swab on Muller Hinton agar plates and dried for 2 to 4 mins. The antibiotic discs were placed on the agar plate by using a disc diffusion dispenser. The antibiotic disc tested were ampicillin (10 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), amoxicillin/clavulanic acid (30 µg), piperacillin/tazobactam (110 µg), and meropenem (10 µg). The plates were then incubated at 37°C for 24 hrs. *Escherichia coli* ATCC 25922 was used as a quality control strain in this study. The diameter of the inhibition zone was measured and recorded to determine the susceptibility level of the selected antibiotics based on the breakpoint recommended by CLSI. The MAR index of the ESBL-producing *E. coli* strains was determined using the formula, a/b, where 'a' refers to the number of antibiotics to which the particular isolate was resistant, and 'b' refers to the total number of antibiotics tested (Krumperman, 1983). The MAR index ≥ 0.2 showed that the bacteria were exposed to a high-risk source of contamination where several antibiotics were used.

2.6 Statistical analysis

The statistically significant differences between the microbial load of ESBL-producing *E. coli* in the vegetable samples and different markets were analysed using the analysis of variance (ANOVA) using IBM SPSS Statistic (ver. 25) software. Furthermore, the significant difference in microbial density between green carol lettuce and mung bean sprouts from different markets were analyzed respectively. A *P*-value <0.05 was considered significant.

3. Results

3.1 Prevalence of ESBL-producing Escherichia coli in vegetables

The incidence and level of ESBL-producing *E. coli* in raw vegetable samples were summarized in Table 2. Of the 180 vegetable samples tested, 113 (62.78%) tested positive for ESBL-producing *E. coli* contamination. Figure 1 shows the PCR assay detection of ESBL-producing *E. coli* with bla_{TEM} in the vegetable samples. Overall, the contamination of ESBL-producing *E. coli* was found to be 73.86% (65/88) in raw vegetables from wet markets and 52.74% (48/92) in hypermarket samples, respectively. The prevalence rate of ESBL-producing *E. coli* in green carol lettuce from wet markets (76.09%) was higher than the green carol lettuce from hypermarkets (48.98%). Similarly, the prevalence rate of ESBL-producing *E. coli* in mung bean sprouts from wet markets was higher (71.43%) than those purchased from hypermarkets (55.81%). Table 2 shows the microbial load of ESBL-producing *E. coli* in raw vegetables from wet markets and hypermarkets. The ESBL-producing *E. coli* microbial load in green carol lettuce from wet markets ranged from <3 to >1100 MPN/g with median

Table 2. Prevalence of ESBL-producing *E. coli* in raw vegetables from wet markets and hypermarkets.

Sampling site	Samples	Number of samples tested (*n*)	Number of positive samples (%)	Microbial load of ESBL-producing *E. coli* (MPN/g)		
				Min	Med	Max
Hypermarket	Green carol lettuce	49	24 (48.98%)	<3.00	3.30	240.00
	Mung bean sprouts	43	24 (57.14%)	<3.00	<3.00	>1100.00
Wet market	Green carol lettuce	46	35 (76.09%)	<3.00	22.00	>1100.00
	Mung bean sprouts	42	30 (71.43%)	<3.00	68.00	>1100.00
Total		180	113 (62.78%)	<3.00	9.20	>1100.00

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22.00 MPN/g, where hypermarkets demonstrated <3 to 240 MPN/g with median 3.30 MPN/g. In mung bean sprouts, the microbial load of ESBL-producing *E. coli* ranged from <3 to 1100MPN/g with median 68.00 and <3.00 MPN/g in wet markets and hypermarkets, respectively. In general, the statistical analysis of the MPN value of ESBL-producing *E. coli* of raw vegetables from wet markets and hypermarkets showed no significant difference (P = 0.353). The MPN values of ESBL-producing *E. coli* in mung bean sprouts showed no significant difference between hypermarkets and wet markets (P = 0.886). However, the prevalence of ESBL-producing *E. coli* in green carol lettuce was significantly higher in wet markets than in hypermarkets (P = 0.014). Among 113 positive samples, 84 harboured bla_{SHV}, and no bla_{CTX-M} was detected in the current study (Table 3). It was shown that bla_{SHV} was the predominant type among the positive samples.

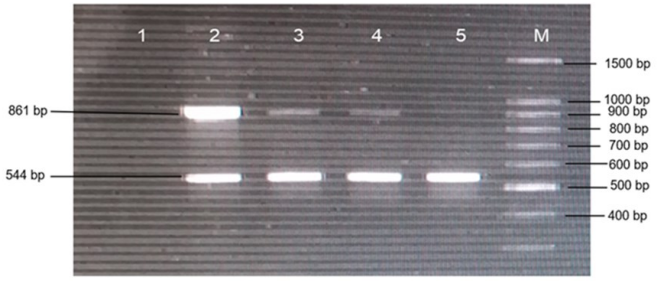

Figure 1. MPN-mPCR of ESBL-producing *E. coli* in samples. Representative amplification of 16S rRNA of *E. coli* (544 bp) and bla_{TEM} (861 bp). Lane M: 100 bp DNA ladder; Lane 2: positive control (*E. coli* with bla_{TEM}); Lane 3 and 4: representative positive samples. Lane 5: negative sample.

Table 3. Dissemination of ESBL genes (bla_{SHV}, bla_{TEM} and bla_{CTX-M}) in positive samples.

Sample	ESBL genes			
	bla_{SHV}	bla_{TEM}	bla_{CTX-M}	$bla_{SHV+TEM}$
Green carol lettuce	37	8	0	14
Mung bean sprouts	47	0	0	5
Total	84	8	0	19

3.2 Antibiotic susceptibility test

A total of 15 ESBL-producing *E. coli* isolates were isolated from positive samples and confirmed by PCR assay. The antibiotic susceptibility profiles of the ESBL-producing *E. coli* isolates were shown in Table 4. Among 15 strains, 12 showed resistance to ampicillin. High resistance rate was found against ciprofloxacin (46.67%), third-generation cephalosporins [cefotaxime (46.67%), ceftazidime (53.33%), ceftriaxone (53.33%)] and aztreonam (60%). All ESBL-producing *E. coli* in the current study were found to be sensitive to cefepime, piperacillin/tazobactam and meropenem. The MAR index of ESBL-producing *E. coli* ranges from 0.1 to 0.6 (Table 4). In the current study, most ESBL-producing *E. coli* strains isolated from green carol lettuce showed a higher MAR index value (0.4 to 0.6) compared to the strains isolated from mung bean sprouts (0.1 to 0.2). MAR index value higher than 0.2 indicated that the isolates might be originated from a high-risk contamination source. The majority of ESBL-producing *E. coli* isolates from raw vegetables were resistant to at least two antibiotics.

4. Discussion

Based on the results, a high prevalence rate of ESBL -producing *E. coli* was discovered in raw vegetables (green carol lettuce, 62.11%; mung bean sprouts, 63.53%). This indicates that the raw vegetables may serve as a route to spread ESBL-producing *E. coli* to the community. Previous studies suggested the consumption of raw vegetables might cause the ingestion of ESBL-producing *E. coli* and facilitate the dissemination of ESBL genes to opportunistic pathogenic bacteria (Reuland *et al*., 2014; van Hoek *et al*., 2015; Hölzel *et al*., 2018).

This study demonstrated a sharp contrast with results from other similar studies that reported low

Table 4. Antibiotics resistance profile of isolated ESBL-producing *E. coli* strains.

No. of isolate	Type of sample	Antibiotic resistance profile	MAR index
11	Mung bean sprouts	AMC	0.1
22	Green carol lettuce	AMP CIP CTX CAZ CRO ATM	0.6
23	Green carol lettuce	CIP CTX CAZ CRO ATM	0.5
24	Green carol lettuce	AMP CIP CTX CAZ CRO ATM	0.6
25	Green carol lettuce	AMP CIP CTX CRO ATM	0.5
26	Green carol lettuce	CAZ CIP AMP CTX	0.4
34	Green carol lettuce	AMP CIP CTX CAZ CRO ATM	0.6
36	Green carol lettuce	AMP CIP CTX CAZ CRO ATM	0.6
37	Green carol lettuce	AMP CRO ATM	0.3
40	Mung bean sprouts	AMC CAZ	0.2
41	Mung bean sprouts	AMP	0.1
45	Mung bean sprouts	AMP CRO ATM	0.3
51	Mung bean sprouts	AMP CAZ	0.2
52	Mung bean sprouts	AMP	0.1
54	Mung bean sprouts	AMP ATM	0.2

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contamination of ESBL-producing *E. coli* in vegetables (Kim *et al*., 2015; van Hoek *et al*., 2015; Kaesbohrer *et al*., 2019). According to Kaesbohrer and colleagues (2019) among 399 vegetable samples, only 1 sample was found positive for ESBL-producing *E. coli*. However, the type of vegetables is not mentioned in the study. In the United Kingdom, Randall *et al*. (2017) reported that no ESBL-producing *E. coli* was detected in vegetable samples purchased from retail stores. On the other hand, the prevalence of ESBL-producing *E. coli* in this study is lower than the study finding in South Africa, which revealed a prevalence rate of 90% (n = 35) in lettuce (Njage and Buys., 2014). The variability in ESBL-producing *E. coli* prevalence rate may be due to the different farming practices, storage methods and hygiene practices of food handlers in different countries.

In general, the prevalence rate of ESBL-producing *E. coli* in vegetables showed no significant difference between wet markets and hypermarkets ($P>0.05$). The results were in concurrence with a study done by Vital *et al*. (2014), which compared the microbial quality of raw vegetables from supermarkets and open-air markets and revealed no significant difference in the microbial density between supermarkets and open-air markets. Furthermore, the present study also compared the microbial load of ESBL-producing *E. coli* of green carol lettuce and bean sprouts from wet markets and supermarkets, respectively. The results revealed that the microbial load of ESBL-producing *E. coli* in green carol lettuce purchased from wet markets was significantly higher than those from hypermarkets ($P<0.05$), however, no significant difference was found between the mung bean sprouts purchased from wet markets and hypermarkets. The inconsistency of the microbial density may be due to the hygienic environment, handling process, packaging, and the holding time of vegetables in different markets (Gizaw, 2019). The green carol lettuces were sold without packaging and displayed in close contact with other types of vegetables. Therefore, it may enhance cross-contamination. Direct contact between the food handlers or customers and fresh produce could lead to cross-contamination in the wet market. Lavilla *et al*. (2008) reported that 27.5% (14/51) ESBL-producing *E. coli* were isolated from food handlers in Spain. In hypermarkets, green carol lettuce was sold in plastic sealed packaging and stored under a cold temperature. Previous studies suggested that low temperatures can reduce bacteria growth in vegetables (Sant'Ana *et al*., 2012; Castro-Ibáñez *et al*., 2017). In addition, Zeng *et al*. (2014) also mentioned that the abuse of storage temperature for vegetables may be a major contributing factor to foodborne outbreaks.

The prevalence rate of ESBL-producing *E. coli* in green coral lettuce is slightly higher than in mung bean sprouts (Table 2). The result was correlated to a study by Blaak *et al*. (2014) suggesting that the presence of ESBL-producing *E. coli* in lettuces reflects the presence of ESBL-producing *E. coli* in cultivation soil as lettuces are soil-grown vegetables. A possible explanation for the higher prevalence rate of ESBL-producing *E. coli* in green carol lettuce may be due to the lettuce being exposed to more reservoirs. Soil and irrigation water are reported as major reservoirs of ESBL-producing *E. coli* (Vital *et al*., 2018; Gekenidis *et al*., 2018). A high density of antimicrobial-resistant bacteria and antibiotic-resistant genes has been found in agricultural soil which is treated with manure (Marti *et al*., 2013; Gao *et al*., 2015). In Tunisia, 7.3% (3/41) and 8.2% (4/49) of ESBL-producing *E. coli* were detected in farming soil and vegetables, respectively (Ben Said *et al*., 2015). Araújo *et al*. (2017) have shown that *E. coli* isolated from vegetables and irrigation water share the same genetic material and antibiotic resistance profile. Gekenidis *et al*. (2018) reported a high occurrence (22%, n = 36) of ESBL-producing *E. coli* in irrigation water from different vegetable farms. Besides, a study conducted by Njage and Buys (2014) reported a high prevalence of ESBL-producing *E. coli* was detected in irrigation water (river, 64%; canal, 73%) and lettuce (90%). In Malaysia, ESBL-producing *Enterobacteriaceae* was detected in urban surface water and irrigation water (89.5%), predominantly *E. coli* and *K. pneumoniae* (Tissera and Lee, 2013). Therefore, the ESBL-producing *E. coli* can adhere and accumulate on the vegetables during the cultivation stage.

Outbreaks of ESBL-producing *E. coli* associated with the consumption of raw vegetables have been reported (Frank *et al*., 2011; Buchholz *et al*., 2011). Allen *et al*. (2010) showed that the consumption of antibiotic resistance *E. coli* may not have an immediate or obvious health outcome for the consumer. However, the transfer of antibiotic-resistant genes by horizontal transfer and conjugation to other bacteria or commensal *E. coli* might take place in the gut (Allen *et al*., 2010). Hence, may lead to the colonization of ESBL-producing bacteria in human guts. Thus, the consumption of a high microbial load of ESBL-producing *E. coli* could pose a health risk to consumers. Previous studies showed a strong correlation between the occurrence of ESBL-producing *E. coli* in food and the incidence of infections (Kaesbohrer *et al*., 2019; Alegría *et al*., 2020). In Malaysia, the nosocomial infections caused by ESBL-producing *E. coli* increased by 8.4% from 2010 to 2016 (Antibiotic Resistance Surveillance Reference Laboratory, Institute for Medical Research, Malaysia, 2017). According to previous studies conducted by Lim *et al*. (2009) and Ho *et al*. (2012), ESBL-producing *E.*

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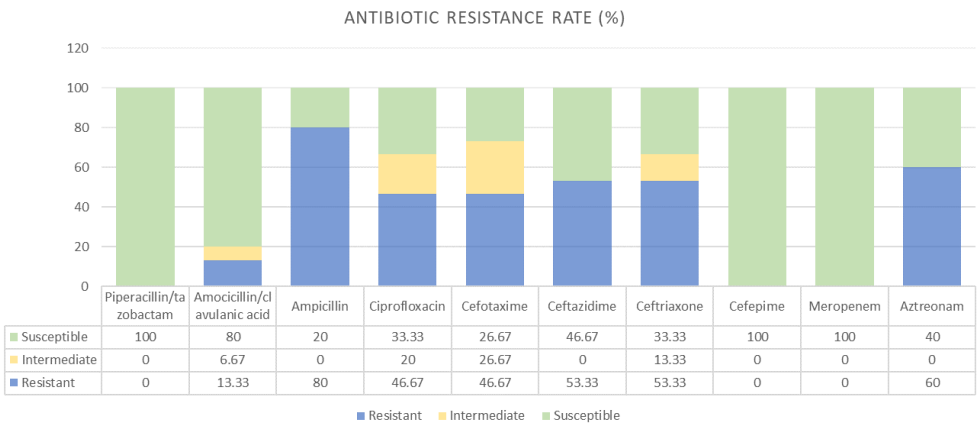
coli was found in the patients of tertiary hospitals in Malaysia. Abubakar *et al*. (2022) reported that ESBL-producing *Enterobacteriaceae* infections correlate with high mortality rates, particularly in patients with diabetes and ICU admissions.

Contrary to Zurfluh *et al*. (2015), no CTX-M type ESBL-producing *E. coli* was detected in the green carol lettuce and mung bean sprouts. The predominant ESBL gene detected in the vegetable samples was bla_{SHV}, followed by bla_{TEM}. However, the present study was comparable to previous studies that reported only SHV-type and TEM-type ESBL-producing *E. coli* were detected in the fresh vegetable sample in Japan (Usui *et al*., 2019) and Italy (Iseppi *et al*., 2018), respectively. The results suggested that the ESBL genotype in vegetables can vary between geographical locations.

The ESBL-producing *E. coli* isolated from green carol lettuce and mung bean sprouts in the present study were found to be high resistance to beta-lactam antibiotics, including ampicillin (80%), cefotaxime (46.67%), ceftazidime (53.33%), ceftriaxone (53.33%) and aztreonam (60%). All ESBL-producing *E. coli* strains showed susceptibility to cefepime, meropenem and piperacillin/tazobactam (Figure 2). Kim *et al*. (2015) reported that in Korea, 15.8% (3/19) ESBL-producing *E. coli* isolated from mixed salads and sprouts showed resistance to ceftazidime and only two isolates were resistant to cefepime. In Morocco, 54% of ESBL-producing *E. coli* isolated from salads were resistant to ceftazidime (Nayme *et al*., 2017). The possible explanation of the different resistance patterns of the ESBL-producing *E. coli* may be due to the strategies and regulations for antibiotics used varying among countries and regions. Based on the results, a total of 60% of the ESBL-producing *E. coli* in this study were multidrug-resistant bacteria (resistant to two or more classes of antibiotics). Apart from beta-lactam antibiotics, the isolated ESBL-producing *E. coli* strains were resistant to monobactam and fluoroquinolones. The results correlated with other researchers who reported that multidrug-resistant are common in ESBL-producing bacteria (Ben Said *et al*., 2015; Abayneh *et al*., 2018).

The ESBL-producing *E. coli* isolates tested in the current study showed MAR indices ranging from 0.1 to 0.6. Based on Table 5, the MAR index value of ESBL-producing *E. coli* isolated from green carol lettuce was higher (0.3 to 0.6) compared to strains from mung bean sprouts (0.1 to 0.3). These findings showed that the majority of the ESBL-producing *E. coli* strains were exposed to high-risk sources where antibiotics are used frequently. Furthermore, the MAR indices of ESBL-producing *E. coli* strain of green carol lettuce were higher than those isolated from mung bean sprouts. Previous studies suggested that soil-grown vegetables have a higher chance of being exposed to antibiotic-resistance bacteria than vegetables grown above the soil (Ruimy *et al*., 2010; Holvoet *et al*., 2013). Thus, different vegetables' growing environments and agricultural activities may cause different antibiotic resistance patterns.

The high prevalence rate of ESBL-producing *E. coli* in vegetables in the current study shows alarming signs to the public. The presence of ESBL-producing *E. coli* in the food chain might pose a significant public health impact. Melzer and Petersen (2007) claimed that the mortality rates of ESBL-producing *E. coli* infections were significantly higher compared to non-ESBL-producing *E. coli.* The overuse and misuse of antibiotics in agriculture and the healthcare system might be the possible risk factor enabling the spread of ESBL-producing *E. coli* in the food chain (Durso and Cook, 2014; Ben Said *et al*., 2015; Yaici *et al*., 2017). Besides that, poor hygiene practices of food handlers may lead to cross-contamination during the processing stage. A previous study reported that ESBL-producing *E. coli* was detected from food handlers (Lavilla *et al*., 2008). Thus, it is important to implement good agricultural practices, proper hygiene practices and biosecurity measures to prevent the continued spread of ESBL-producing *E. coli* throughout the vegetable production chain.



	Piperacillin/tazobactam	Amocicillin/clavulanic acid	Ampicillin	Ciprofloxacin	Cefotaxime	Ceftazidime	Ceftriaxone	Cefepime	Meropenem	Aztreonam
Susceptible	100	80	20	33.33	26.67	46.67	33.33	100	100	40
Intermediate	0	6.67	0	20	26.67	0	13.33	0	0	0
Resistant	0	13.33	80	46.67	46.67	53.33	53.33	0	0	60

Figure 2. Antibiotic susceptible profile of ESBL-producing *E. coli* in raw vegetables.

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5. Conclusion

The study revealed the high prevalence of ESBL-producing *E. coli* in green carol lettuce (62.11%) and mung bean sprouts (63.53%) in retail markets in Malaysia. The ESBL-producing *E. coli* density in the samples ranged from <3 to >1100 MPN/g with median 9.2 MPN/g. Thus, raw vegetables could act as a possible vehicle to transmit ESBL-producing *E. coli* to humans. The occurrence of multidrug-resistant bacteria in vegetables is a sign of alarm as it may lead to critical clinical issues. A total of 60% of the isolated ESBL-producing *E. coli* showed multidrug resistance. The highest MAR index value of the ESBL-producing *E. coli* strains was 0.6. Therefore, good agricultural practices should be employed in the early stage of food production to avoid the dissemination of antibiotic-resistant bacteria in the environment and human. Besides, a continuous monitoring program of the antibiotic-resistant pattern of ESBL-producing *E. coli* and food pathogens at the national level is important to control the spread of multidrug-resistant ESBL-producing *E. coli* and ensure food safety.

Conflict of interest

The authors declare no conflict of interest.

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