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INCIDENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ENTEROBACTERIACEAE IN RAW VEGETABLES SOLD IN DISTRICT KOHAT

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ABSTRACT

Background: Extended spectrum *β*-lactamase (ESBL) producing bacteria is a growing public health concern globally because it shows resistance to *β*-lactam antibiotics. The consumption of contaminated food products, particularly raw vegetables, is a significant contributing factor to the spread of these bacteria. This study investigates the occurrence of ESBL producing bacteria in raw vegetables retailed in the District of Kohat. 80 Samples of raw vegetables were collected from wholesale markets, retail shops and small vendors in the District of Kohat, and the bacterial strains were identified as *E. coli* and *Klebsiella pneumoniae.*

Materials and Methods: Colony-forming unit (CFU) estimation, Gram staining, biochemical testing, most probable number (MPN) analysis, and the double disc synergy test to detect ESBL production were some of the procedures included in the study approach.

Results: The coliform counts in raw vegetables were found to range from 25-45×10^6 CFU/g. Different commercially available antibiotics were used to determine their potency against the tested bacterial isolates (*E. coli* and *K. pneumoniae*). *Klebsiella pneumoniae* displayed resistance against Ampicillin AP (10µg) and Cefoxitin FOX (30 µg) antibiotics. In contrast, *E. coli* showed sensitivity to all antibiotics except Cefoxitin FOX (30 µg). Additionally, the study also estimated the Most Probable Number (MPN) value of coliforms in raw vegetables. The MPN value provides an estimate of the number of viable bacteria present in a sample. In this study, the highest MPN value was recorded in Tomato (T 1) having MPN index per 100 mL was 33, while the lowest MPN value was for Lettuce (L 3) having MPN index per 100 mL was 4. The high MPN value indicates a higher risk of contamination with fecal matter.

Conclusion: In conclusion, our study identified the presence of ESBL producing *E. coli* and *Klebsiella pneumoniae* in raw vegetables retailed in the District of Kohat. Among these, *E. coli* was detected in 12 samples (15%) while *Klebsiella pneumoniae* was detected in 8 samples (10%). The antibiotic resistance of these bacteria suggests a potential risk to public health. Therefore, it is essential to implement proper food safety practices programs to prevent the spread of these bacteria.

INTRODUCTION

Enterobacteriaceae that produce extended spectrum beta-lactamase (ESBL) enzymes are a major public health problem due to their capacity to degrade a wide variety of beta-lactam medicines, including third generation cephalosporin (Akpaka *et al.*, 2021). These bacteria have emerged as a major source of infections in hospitals and the general population, increasing disease, fatalities, and medical costs. When treating patients in the community and in hospitals, the ESBL enzymes have grown to be a significant barrier for healthcare professionals, because *β*-lactamases share their ability to hydrolyze third generation cephalosporin and aztreonam, and it can be killed by clavulanic acid (Rawat & Nair, 2010). As a result, the prevalence of bacterial infections is increasing over time. The National Health Service (NHS) reported that *E. coli* O157 causes infections such as bloody diarrhea, severe stomach pain, and kidney failure, and it is usually found in the feces and gut of many animals (NHS, 2023). Raw leafy vegetables contaminated with *E. coli* always require washing before adding to salads unless they are prepared or labeled as ready to eat. *Klebsiella pneumoniae* is a gram-negative bacterium that generally causes nosocomial infections such as surgery or wound infections, bloodstream infections, pneumonia, and meningitis, according to a study by (Ashurst & Dawson, 2018). Due to the rise in antibiotic resistance among hospital pathogens, nosocomial infections, also known as hospital-acquired illnesses, are a significant health problem. Infections acquired in hospitals are typically brought on by ESBL-producing bacteria, according to 2009 research by Falagas and Karageorgopoulos. In addition to lowering the quality of raw veggies, ESBL-producing bacterial strains have been linked to community-related diseases (Zurfluh *et al.*, 2015). Another investigation by (Colosi *et al.*, 2020) revealed the finding of a predominance of Enterobacteriaceae isolates generating extended spectrum beta-lactamase (ESBL) from retailed raw vegetables. Due to their potential effects on public health, the rise and abundance of ESBL-producing bacteria in the food chain have come under increasing scrutiny. Human consumption of raw vegetables made up between 10% and 12.3% of total vegetable intake in 2022 (CDC, 2022). The consumption of raw vegetables is viewed globally as crucial for obtaining essential vitamins such as Vitamin B and water-soluble Vitamin C (Victoria Frankel, 2022).

Adequate consumption of food, including vegetables, helps protect against chronic diseases such as cancer, obesity, cardiovascular diseases, diabetes, and metabolic syndromes, while also improving related risk factors (Ülger *et al.*, 2018). According to Shayna Komar, a licensed and registered dietitian, incorporating raw vegetables and fruits into daily dietary routines offers numerous health advantages, such as improved digestion, enhanced skin health, increased energy levels, and optimized metabolic activities (Piedmont, 2022). The preparation of raw vegetables is simple and aids in weight management, ensuring individuals stay fit and healthy. Compared to overcooked food, raw food items have garnered attention due to their higher nutritional levels, as overcooking vegetables can diminish their nutritional value and disrupt their food structure (Brookie *et al.*, 2018). In fact, raw vegetables and fruits have been found to be more effective in promoting mental health compared to the consumption of processed food products. They have been associated with reduced depressive symptoms, improved life satisfaction, and enhanced positive moods (Brookie *et al.,* 2018). Furthermore, incorporating raw vegetables into one's daily diet has been linked to weight loss, reduced risks of cancer and diabetes, cardiovascular health improvement, enhanced skin appearance, improved digestion, elevated energy levels, and stabilized blood glucose levels (Diabetes UK, 2019). Regular consumption of raw vegetables also strengthens the immune system and aids in disease prevention and infection control. Carrots and cucumbers, for example, are frequently used in salads due to their high carotenoid content. Carotenoids have been found to reduce the risk of anemia and promote the production of red blood cells. Cucumbers, on the other hand, are rich in phytonutrients such as lignin, flavonoids, and triterpenes, which possess anticancer, antioxidant, and anti-inflammatory properties (Anata, 2021). Lettuce, known for its beta-carotene content (Vitamin A), is essential for bone, skin, and eye health, and it also provides folate, which promotes the health of babies during pregnancy and helps prevent neural tube defects (Government of Northwest Territories, 2022).

However, there are several factors that can affect the quality of vegetables. One crucial aspect is the knowledge and skills of farmers in irrigation and ensuring the quality of their yield. A research study by (Stoleru *et al.,* 2020) suggests that optimal vegetable yields depend on mineral fertilizers, herbicides, antioxidant compounds, and residual biomass valorization. These factors contribute to improved production outcomes. However, there are other factors that can negatively impact vegetable quality. These include a lack of training among farmers, cross-contamination during food handling, the use of fresh fish or chicken in vegetable salads, and the practice of leaving vegetables uncovered and exposed to ambient air during sale (Toe *et al.*, 2017). This research study aims to examine the incidence of ESBL-producing bacteria in raw vegetables within the retail sector of District Kohat, Pakistan. The industrial sector of District Kohat has experienced a 5.02% increase compared to the previous year, focusing on human resources and raw materials for economic growth (Umer *et al.*, 2020). However, there is no literature available about the current study, as they work to increase their economic growth in District Kohat by increasing the flexibility and quality of their produce, raw materials, and human resources, the food and retail raw vegetable businesses can profit from the current research.

MATERIALS AND METHOD

Sample Collection

A total of 80 raw vegetable samples (n = 80) were procured from wholesale markets, retail shops, and small vendors in District Kohat. Each sample category, including tomatoes, cucumbers, cabbage, onions, carrots, lettuce, spinach, and radish, was represented by 10 samples. To ensure utmost sterility and minimize the risk of contamination, the samples were aseptically collected and placed in sterile plastic zip lock bags. These bags were properly labeled with information regarding the vegetable type, source, and collection date. To maintain sample integrity, they were promptly transported within two hours of collection, utilizing cold boxes maintained at a temperature of 4°C, to the Department of Microbiology.

Isolation of Bacteria from Raw Vegetables

The process of isolating microorganisms from the collected samples involved the use of pure culturing techniques. Isolation, enumeration, and maintenance of pure cultures were performed exclusively on MacConkey's agar media.

Sample Processing Utilizing the Serial Dilution Method

Each vegetable sample was carefully cut into 10 grams of small pieces under sterile circumstances, and then the pieces were combined to make a paste. Then, 90 mL distilled water was used to dissolve this material. The conventional plating technique was used to create serial dilutions up to 10^6. 1 mL of the inoculum was put onto sterile petri plates containing selective media (MacConkey agar) and distributed equally across the surface of the medium for each of the dilutions (10^1, 10^3, and 10^5). The plates were then incubated for 24 to 48 hours at a temperature of 37°C. After incubation, the number of colonies that formed on the media's surface was counted using a colony counter, and the CFU was then calculated using the CFU formula.

Bacterial Identification

Traditional microbiological methods, such as Gram staining and examination of colony characteristics, were used to identify the bacterial species present in the raw vegetable samples. To discriminate between Gram-positive and Gram-negative bacteria after obtaining pure colonies, Gram staining was carried out. This is an essential first step in the identification of bacteria.

Biochemical Identification

To biochemically identify bacterial colonies, a range of biochemical techniques including the IMViC test, sugar fermentation test, catalase test, and oxidase test were utilized (Cheesbrough, 2005).

Most Probable Number (MPN)

The total coliform count in food or water samples was determined using the most probable number (MPN) technique. Vegetable sample testing was carried out in an aseptic manner. There were three phases in the MPN method.

Presumptive Test

The presumptive test provided the strongest evidence that Gram-negative coliform bacteria were present in the samples. Coliform bacteria were grown in nutrient broth, and gas production was detected using an inverted Durham's tube. Turbidity and gas production were suggested coliform bacteria were present in the samples. Each vegetable sample received five tubes of double- concentration media and ten tubes of single-concentration medium. With the help of pipette the food samples were inoculated in each tubes respectively, which were then incubated at 37°C for 24-48 hours. Following incubation, the number of bacteria present in the sample was calculated by comparing the positive response tubes to a reference chart. Turbidity in the growing media and the production of 10% or more gas in the Durham tube within 24 to 48 hours indicated a positive presumptive test for coliform bacteria and possible fecal contamination. Following that, a standard MPN table was used to count the number of coliform bacteria in 100 mL of the sample (Phyo *et al.*, 2019).

Confirmed Test

To ascertain if the coliforms were indicator bacteria of fecal origin, especially *Escherichia coli*, positive samples from the presumptive test were chosen for the confirmed test. *E. coli* was distinguished from other Gram-negative coliform bacteria using Eosin Methylene Blue (EMB) medium by looking at the formation of a greenish metallic sheen. After 24 hours of incubation at 37°C, colonies exhibiting colour indication were discovered by streaking a loopful of the material from positive tubes (Phyo *et al.*, 2019).

Complete test

A Durham's tube was used to inoculate bacterial colonies from the verified test's EMB medium into nutritional broth at 37°C. Gas generation and turbidity in the broth media were indicators of the presence of fecal indicator *E. coli* (Phyo *et al.*, 2019).

Determination of ESBL-Producing Bacteria

ESBL generation was suspected in Enterobacteriaceae isolates that shown resistance to or diminished sensitivity to the screening indicator cephalosporin (cefotaxime and/or ceftazidime). For confirmation of ESBL generation, isolates having inhibition zone sizes of ≤ 27 mm for cefotaxime (30 µg) and/or ≤ 22 mm for ceftazidime (30 µg) were chosen as ESBL producers (Kebede *et al.*, 2022).

Gram-negative bacteria were standardized using a 0.5 McFarland solution and dispersed using a sterile cotton brush on Mueller Hinton Agar (MHA) plates to confirm ESBL development. A disc of Cefotaxime (CTX 30 µg) was positioned 20 mm (center to center) apart from the disc of Augmentin (amoxicillin/clavulanic acid) and placed on the MHA's surface. The plates were next incubated for 24 hours at 37°C. The extension of the inhibition zone edge of the cefotaxime disc towards the disc containing Amoxicillin-Clavulanic acid was recorded as a positive result for ESBL production (Oyeyipo *et al.*, 2021).

Antibiotic Susceptibility Test

The Clinical and Laboratory Standard Institute (CLSI) recommendations were followed for performing the antibiotic sensitivity test. MHA plates were inoculated using a suspension of ESBL-producing bacterial isolates. On the surface of the infected culture plates, several antibiotic discs were positioned, including Ampicillin (AP 10 µg), Meropenem (MEM 10 µg), Chloramphenicol (C 30 µg), Gentamicin (CN 16 µg), Sulfamethoxazole (TS 25 µg), Cefotaxime (CTX 30 µg), and Cefoxitin (FOX 30 µg). To find out how susceptible the ESBL-producing bacteria were to the antibiotics, the plates were incubated at 37°C for 24 hours. The inhibitory zones' length was then measured in millimeters (Romyasamit *et al.*, 2021).

RESULTS

Determination of Colony Forming Unit (CFU)

Using the serial dilution approach, several vegetable samples were obtained from retail outlets. The culture plates were then poured, followed by a 24-hour incubation period at a temperature of 37°C. Following the incubation time, a colony counter was used to count the number of colonies that were visible on the media's surface. The following formula was then used to calculate the CFU values:

$$\frac{CFU}{g} = \frac{No. of\ colonies\ \times Dilution\ Factor}{Volume\ of\ sample\ inoculated}$$

Upon calculation, the total coliform counts in raw vegetables were found to range from 25-45×10^6 CFU/g. The vegetable sample with the highest coliform count was Tomato1, with a count of 45×10^6 CFU/g. On the other hand, Lettuce3 exhibited the lowest coliform count of 25×10^6 CFU/g, as illustrated in Table 4.1. The higher CFU values may indicate a greater risk of fecal contamination and the potential presence of pathogens in the veggies samples.

Table 1: Total coliform counts of bacteria in raw vegetables (×10^6 CFU/g)

Vegetables code	Bacterial isolates	TCC (×10^6 CFU/g)	Vegetables code	Bacterial isolates	TCC (×10^6 CFU/g)
T1	*KP*	45	**S2**	*EC*	29
T2	*KP*	32	**S3**	*EC*	38
T3	*KP*	36	**C1**	*KP*	34
T4	*KP*	29	**C2**	*EC*	45
Cu1	*EC*	38	**C3**	*EC*	41
Cu2	*EC*	40	**C4**	*EC*	37
Cu3	*KP*	36	**L1**	*KP*	44
Cu4	*EC*	30	**L2**	*EC*	31
Cu5	*EC*	34	**L3**	*EC*	25
S1	*KP*	32	**Cab1**	*EC*	36

Key: T=Tomato, Cu=Cucumber, S=Spinach, C=Carrots, L=Lettuce, Cab=Cabbage, EC= *E. coli*, KP= *K. pneumonia*

Morphological and biochemical Identification of isolated bacteria from raw vegetables

In this study, different raw vegetable samples including tomatoes, cucumbers, cabbage, onions, carrots, lettuce, spinach, and radish were collected from different sites and subjected to microbiological analysis. The samples were cultured on selective media (MacConkey's agar), and suspected bacterial isolates were obtained. The bacterial isolates were then subjected to various tests, including Gram staining and biochemical tests, to identify potential ESBL-producing Enterobacteriaceae in raw veggies. Based on Gram staining and biochemical profile two isolates were obtained such as *E. coli* and *Klebsiella pneumoniae,* both of which are known to be common ESBL-producing Enterobacteriaceae.

Table 2: Gram staining and biochemical profile of bacteria isolated from waste irrigating water and raw vegetables.

Biochemical Identification												
Bacterial isolates	**Gram staining**	Catalase	Oxidase	Indole	Methyl red	Voges Proskauer	Simmons Citrate	Glucose	Sucrose	Lactose	Maltose	Mannitol
E. coli	-R	+	-	+	+	-	-	+	+	+	+	+
Klebsiella pneumoniae	-R	+	-	-	-	+	+	+	+	+	+	+

Key: + = positive, - = negative, -R = negative rods

Table 3: Presumptive Test Chart of MPN

Vegetables Type	No. of tubes giving a positive reaction			MPN (per 100ml)	95% Confidence Limits	
5 of 10 ml		**5 of 1 ml**	**5 of 0.1 ml**		**Lower**	**Upper**
T1	4	3	1	**33**	9	78
T2	3	2	1	**17**	7	40
T3	3	0	0	**8**	2	22
T4	2	2	0	**9**	2	21
Cu1	4	2	1	**26**	7	67
Cu2	2	1	1	**9**	2	21
Cu3	3	0	0	**8**	2	22
Cu4	2	1	0	**7**	1	17
Cu5	2	1	0	**7**	1	17
S1	3	3	0	**17**	7	40
S2	2	1	1	**9**	2	21
S3	3	0	0	**8**	2	22
C1	2	3	0	**12**	3	28
C2	3	0	0	**8**	2	22
C3	2	1	1	**9**	2	21
C4	2	1	0	**7**	1	17
L1	1	1	1	**6**	1.8	15
L2	3	1	1	**14**	6	36
L3	1	1	0	**4**	0.7	12
Cab1	3	1	0	**11**	5	35

Key: T= Tomato, Cu= Cucumber, S= Spinach, C= Carrots, L= Lettuce, Cab= Cabbage

In Presumptive Test, the MPN Index per 100 mL for combinations of positive and negative results when five 10 mL, five 1 ml, and five 0.1 mL portions of samples were used. In this study the highest MPN per 100 mL for coliform count was in Tomato (T 1) having MPN index per 100 mL was 33, while the lowest MPN value in this study was for Lettuce (L 3) having MPN index per 100 mL was 4.

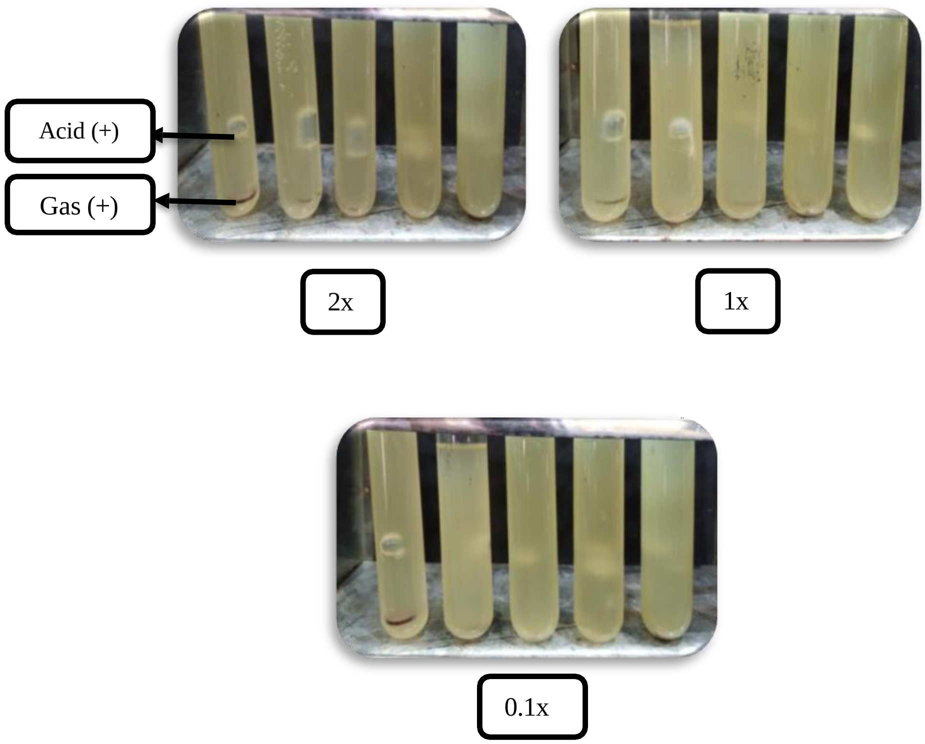

Acid (+)
Gas (+)
2x
1x
0.1x

Table 4: Confirmed Test of MPN

Growth on EMB	Production of Greenish Metallic Sheen	Result
+	+	Non-potable

Key: + = positive, EMB = Eosin methylene blue

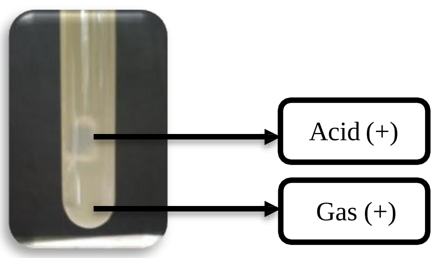
From the presumptive test, the fecal bacteria was inoculated on Eosin methylene blue agar (EMB), after incubation suspected growth on EMB indicated positive result as shown in Table 4.4, it suggests that all the positive tubes of presumptive test were recorded as positive on EMB and thus the food items were not potable.

Table 5: Complete Test of MPN

Isolates test	Result	Probability
Lactose broth (+/-)	+	Non-potable
Reaction/Morphology	Gram –ve, Rod	Non-potable

Key: + = positive, - = negative

In complete test of MPN, the bacteria from EMB agar again inoculated in Nutrient Broth. Following incubation, the turbidity and gas production in Durham tubes indicates the positive reaction. It suggests that all the isolates reported as positive and the food samples were not potable.

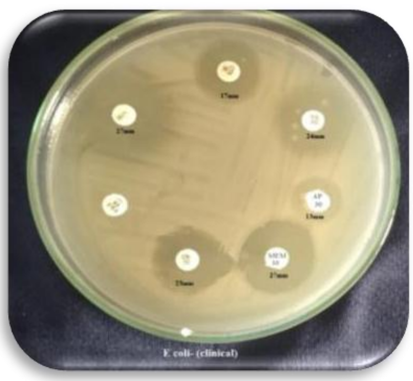


Antibiotic Sensitivity Test

Different commercially available antibiotics were used to determine their potency against tested bacterial isolates (*E. coli* and *K. pneumoniae*) on MHA medium. It was observed that the *E. coli* displayed sensitivity against all the antibiotics except Cefoxitin (FOX 30), on the other hand *K. pneumoniae* also show sensitivity against all the antibiotics except Ampicillin (AP 10) and Cefoxitin (FOX 30) as shown in Table 4.5

Table 6: Average Zone of Inhibitions (mm ± STDEV) of *E. coli* and *K. pneumoniae.*

S#	Antibiotics	*E. coli*	*K. pneumoniae*
1	**Cefotaxime CTX (30 µg)**	17.2 ± 0.25	25.2 ± 0.25
2	**Meropenem MEM (10µg)**	27.3 ± 0.26	15.1 ± 0.15
3	**Chloramphenicol C (30 µg)**	27.1 ± 0.28	28.4 ± 0.11
4	**Gentamicin CN (16 µg)**	23.26 ± 0.25	17.4 ± 0.11
5	**Ampicillin AP (10 µg)**	13.23 ± 0.25	00 ± 00
6	**Sulfamethoxazole TS (25 µg)**	24.2 ± 0.25	23.2 ± 0.25
7	**Cefoxitin FOX (30 µg)**	00 ± 00	00 ± 00



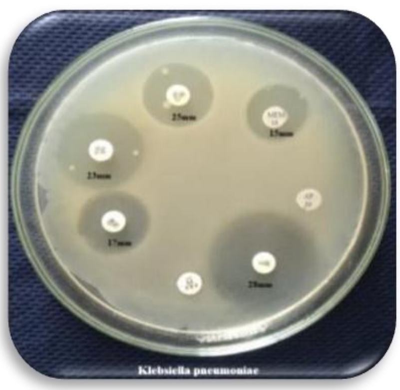


Figure 1: Susceptibility pattern of the isolated bacteria (*E. coli*) against different antibiotics

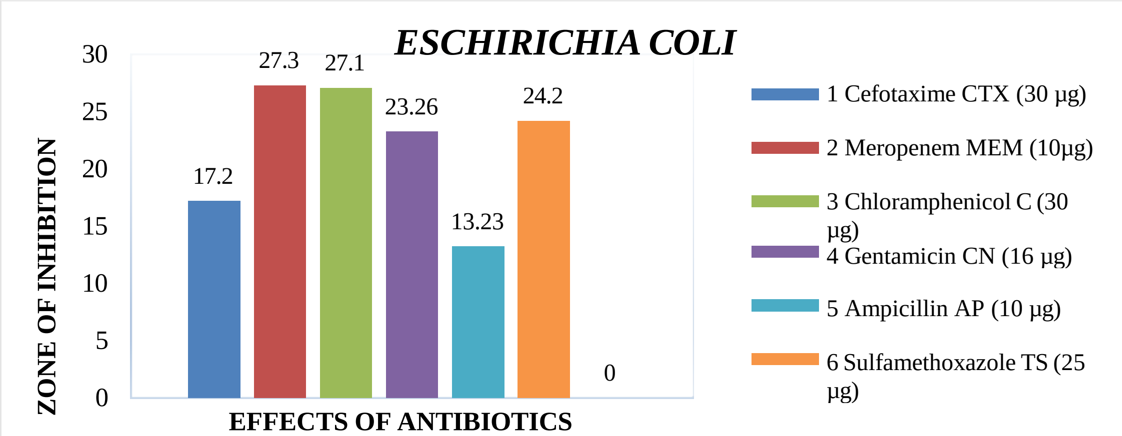
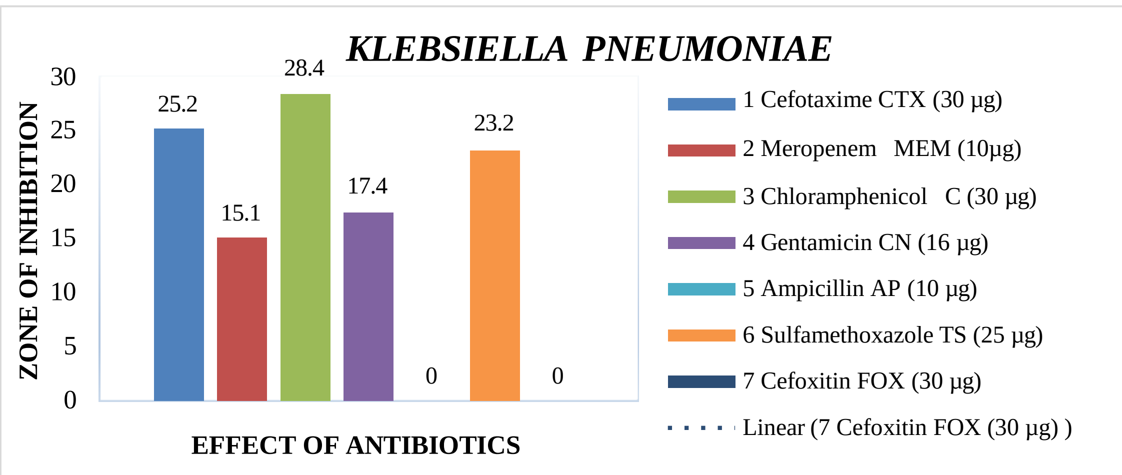
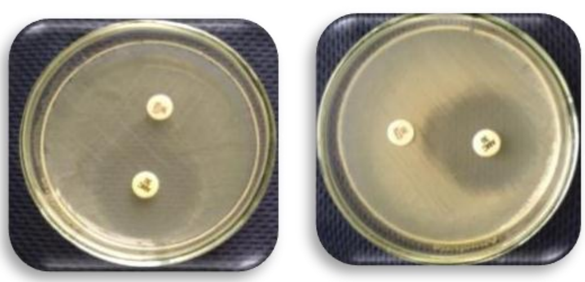


Figure 2: Susceptibility pattern of the isolated bacteria (*K. pneumoniae*) against different antibiotics



ESBL Double Disc Synergy Test (DDST) by using Cefotaxime (CTX) disc and Augmentin (AMC) disc

The extension of the inhibition zone edge of the cefotaxime disc towards the disc containing Amoxicillin-Clavulanic acid was recorded as a positive result for ESBL.



Prevalence of ESBL producing bacterial isolates from raw vegetables

The Table 7 summarizes the prevalence of ESBL-producing *E. coli* (EC) and *Klebsiella pneumoniae* (KP) isolates. A total of 80 samples were collected from retailing sectors. Among these samples, 12 (15%) were identified as ESBL-producing *E. coli* in a variety of raw veggies, including cucumber, spinach, carrots, lettuce, and cabbage. And 8 (10%) were identified as ESBL-producing *Klebsiella pneumoniae* in a variety of raw veggies, including tomato, cucumber, spinach, carrots and lettuce. Overall, 20 samples (25%) showed the presence of ESBL-producing isolates. The prevalence of *E. coli* was found to be higher. No ESBL-producing bacterial isolates were found in onion and radish. Six out of the eight edible raw vegetable samples included high concentrations of ESBL-producing *E. coli* and *K. pneumoniae*, which were more commonly detected in tomato, cucumber and carrot samples than in other raw veggies sample.

Table 7: Prevalence of ESBL-producing bacteria isolated from raw vegetables

Origin	**N**	**No. of ESBL-producer bacterial isolates (%)**		
		EC	**KP**	**Total**
Tomato	10	00	4 (40%)	4 (40%)
Cucumber	10	4 (40%)	1 (10%)	5 (50%)
Spinach	10	2 (20%)	1 (10%)	3 (30%)
Onion	10	00	00	00
Carrots	10	3 (30%)	1 (10%)	4 (40%)
Lettuce	10	2 (20%)	1 (10%)	3 (30%)
Radish	10	00	00	00
Cabbage	10	1 (10%)	00	1 (10%)
Total	**80**	**12 (15 %)**	**8 (10 %)**	**20 (25%)**

Key: N= number, ESBL= extended spectrum β-lactamase, EC= *E. coli*, KP= *Klebsiella pneumoniae*

Figure 3: Prevalence of *E. coli* from raw vegetables

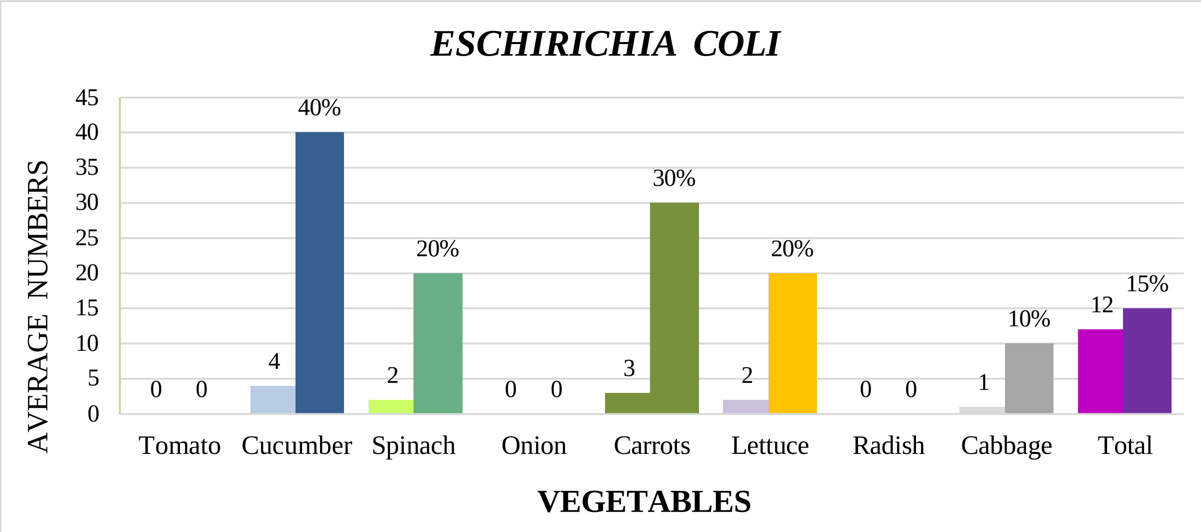
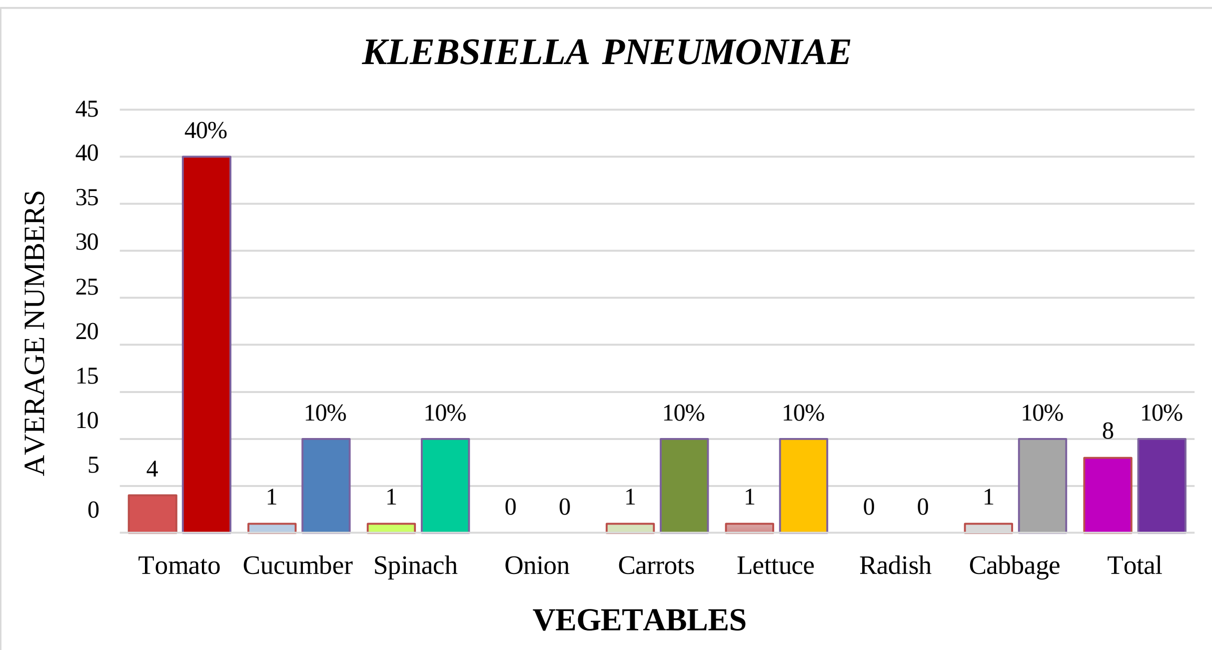


Figure 4: Prevalence of *K. pneumoniae* from raw vegetables



DISCUSSION

According to the study, there is a sizable incidence of Enterobacteriaceae that produce ESBLs in raw vegetables marketed in the Kohat District. 25% of the 80 samples that were obtained tested positive for Enterobacteriaceae that produce ESBLs. This conclusion is alarming since it suggests a possible harm to the general public's health. Because Enterobacteriaceae strains include ESBLs, it is possible that they are resistant to standard antibiotics, making it challenging to treat illnesses brought on by these bacteria. Several variables may be responsible for the increased prevalence of Enterobacteriaceae that produce ESBL in raw veggies. First, these bacteria could be introduced to the veggies during the growing phase by using polluted irrigation water or sewage in agricultural practices (Gekenidis *et al.,* 2020).

Several studies have looked at the presence of Enterobacteriaceae that produces ESBLs in various foods, including raw vegetables (Blaak *et al.,* 2014). The results of this investigation support earlier findings, emphasizing the pervasiveness of these antibiotic-resistant microorganisms in food. The public's health is seriously endangered by Enterobacteriaceae that produce ESBLs in raw veggies. Antibiotic-resistant bacteria (ARB) can spread to humans through the consumption of infected veggies (Hölzel *et al.,* 2018). ESBL-producing Enterobacteriaceae infections are linked to higher rates of morbidity, death and medical expenses (Sabino *et al.,* 2019). The issue is made worse by the few available treatments for these illnesses. As a result, it is essential to address this problem as soon as possible and put policies in place to maintain food safety and reduce the spread of antibiotic-resistant bacteria across the food chain.

In this study, colony forming unit (CFU) procedure was also employed to count the bacterial load in raw veggies. Upon calculation, the total coliform counts in raw vegetables were found to range from $25\text{-}45\times10^6$ CFU/g. The vegetable sample with the highest coliform count was Tomato (T 1), with a count of 45×10^6 CFU/g. On the other hand, Lettuce (L 3) exhibited the lowest coliform count of 25×10^6 CFU/g. It confirms the findings of (Orji *et al.,* 2017) who proclaimed higher bacterial count of 10^6 CFU/g in salad vegetables. (Erkmen and Bozoglu, 2016) also described that the bacteria count in veggies may ranges from $10^2\text{-}10^7$ CFU/g.

Bacterial isolates from raw veggies like *E. coli* and *K. pneumoniae* were identified using biochemical assays. Both *E. coli* and *K. pneumoniae* had a positive response in the catalase test, indicating their existence. Additionally, both species results from the oxidase test were negative, corroborating the identification of both. The indole test revealed that *K. pneumoniae* and *E. coli* had different characteristics since it detected *E. coli* but not *K. pneumoniae*. The methyl red test yielded positive results for the *E. coli* but negative results for *K. pneumoniae*. Results from the Voges-Proskauer test indicated that *K. pneumoniae* was reacted positively to whereas *E. coli* was negatively reacted to. The Simmons Citrate test confirmed the presence of *K. pneumoniae* and ruled out *E. coli*. Finally, the glucose, sucrose, lactose, maltose, and mannitol tests were positive for both *E. coli* and *K. pneumoniae*, indicating their ability to utilize these sugars as energy sources.

To evaluate the amounts of fecal contamination in the food samples, the MPN index was developed (Doğan-Halkman *et al.,* 2003). In this study, Tomato (T 1) had the greatest fecal contamination per 100 mL with a value of 33, whereas lettuce (L 3) had the lowest contamination with a value of 4. These findings draw attention to the possible health hazards of eating raw vegetables since high levels of fecal contamination during preparation and distribution point to unhygienic conditions. All the presumptive tests conducted on the bacterial isolates were positive when grown on Eosin Methylene Blue (EMB) media, indicating the presence of fecal coliforms. This confirms the earlier findings of fecal contamination in the raw vegetable samples. For the complete test the bacterial colonies on EMB medium when introduced into Nutrient Broth using Durham tubes, the emergence of obvious turbidity and the emission of gas indicated the existence of fecal coliforms after incubation.

For the double disc synergy test (DDST), twenty (20) bacterial isolates in raw vegetables were positive, this is the case with the Cefotaxime (CTX), which displayed a sharp extension towards the Augmentin disc (amoxicillin/clavulanic acid), it confirms the findings of (Oyeyipo *et al.*, 2021). In addition, different commercially available antibiotics were used to determine their potency against the tested bacterial isolates (*E. coli* and *KP*). *Klebsiella pneumoniae* displayed resistance against Ampicillin AP (10µg) and Cefoxitin FOX (30 µg) antibiotics. In contrast, *E. coli* showed sensitivity to all antibiotics except Cefoxitin FOX (30 µg). The highest activity was observed for *E. coli* against Meropenem MEM (10 µg) with a value of 27.3 ± 0.26, while *Klebsiella pneumoniae* displayed the highest activity against the Chloramphenicol C (30µg) antibiotic with a value of 28.4 ± 0.11. Conversely, *E. coli* exhibited the lowest activity against Cefoxitin FOX (30 µg) with a value of 0 ± 0, whereas *Klebsiella pneumoniae* showed the lowest activity against Ampicillin AP (10 µg) and Cefoxitin FOX (30 µg) antibiotics with values of 0 ± 0.

It was determined how frequently ESBL-producing bacterial isolates were present in raw veggies. This study examined 80 samples of vegetables, with 10 samples each of tomatoes, cucumbers, spinach, onions, carrots, lettuce, radishes, and cabbage. Among these, *E. coli* was detected in 12 samples (15%) while *Klebsiella pneumoniae* was detected in 8 samples (10%). Twenty samples in all (25%) were discovered to have ESBL-producing bacterial isolates. These findings show a high incidence of ESBL-producing bacteria in raw veggies, which might be dangerous for humans.

CONCLUSIONS

Our study sheds light on the incidence of ESBL-producing Enterobacteriaceae in raw vegetables sold in the Kohat area. The results highlight the need to put precautionary measures in place, keeping an eye on trends of antibiotic resistance, and raising public awareness to reduce any possible problems brought on by these bacteria. It is possible to protect the public's health and lessen the effects of antibiotic resistance in the community by taking a multidisciplinary approach and following the suggestions. To stop the transfer of these bacteria to veggies it is necessary to use clear water for irrigation. Moreover, there is a necessity for public awareness campaigns and educational programs to promote proper hygiene practices among consumers, including thorough washing of raw vegetables before consumption. It is crucial to emphasize the role of individuals in preventing the transmission of ESBL-producing bacteria and other antibiotic-resistant pathogens through responsible food handling and personal hygiene. Furthermore, It is necessary to conduct more research to fully understand the genetic processes that give rise to the isolated strains' production of ESBL.

REFERENCES

Akpaka, P. E., Vaillant, A., Wilson, C., & Jayaratne, P. (2021). Extended spectrum beta-lactamase ESBL produced by gram-negative bacteria in Trinidad and Tobago. *International Journal of Microbiology*, 2021.

Anata, (2021). What are the benefits of carrot and cucumber? [online]. Available at: https://www.anata.in/blogs/faq/what-are-the-benefits-of-carrot-and-cucumber#:~:text=The%20carotene%20present%20in%20carrots,inflammatory%20and%20anti%2Dcancer%20benefits. [accessed: 22nd February 2023]

Ashurst, J. V., & Dawson, A. (2018). *Klebsiella pneumonia.*

Blaak, H., van Hoek, A. H., Veenman, C., van Leeuwen, A. E. D., Lynch, G., van Overbeek, W. M., & de Roda Husman, A. M. (2014). Extended spectrum ß-lactamase-and constitutively AmpC-producing Enterobacteriaceae on fresh produce and in the agricultural environment. *International journal of food microbiology*, 168, 8-16.

Brookie, K. L., Best, G. I., & Conner, T. S. (2018). Intake of raw fruits and vegetables is associated with better mental health than intake of processed fruits and vegetables. *Frontiers in psychology*, 487.

CDC, (2022). Only 1 in 10 adults eating enough fruits and vegetables, CDC says [Online]. Available at: https://thehill.com/changing-america/well-being/longevity/588767-only-1-in-10-adults-eating-enough-fruits-and-vegetables/#:~:text=The%20CDC%20found%20that%20only%2010%20percent%20of%20adults%20were,the%20recommended%20servings%20of%20fruit. [Accessed: 16th February 2023]

Cheesbrough, M. (2005). *District laboratory practice in tropical countries, part 2*. Cambridge university press.

Colosi, I. A., Baciu, A. M., Opriș, R. V., Peca, L., Gudat, T., Simon, L. M., ... & Costache, C. (2020). Prevalence of ESBL, AmpC and carbapenemase-producing enterobacterales isolated from raw vegetables retailed in Romania. *Foods*, *9*(12), 1726.

Diabetes UK, (2019). Raw Food Diet [online]. Available at: https://www.diabetes.co.uk/diet/raw-food-diet.html [accessed: 21st February 2023]

Doğan-Halkman, H. B., Çakır, İ., Keven, F., Worobo, R. W., & Halkman, A. K. (2003). Relationship among fecal coliforms and *Escherichia coli* in various foods. *European Food Research and Technology*, 216, 331-334.

Erkmen O, Bozoglu TF. Spoilage of Vegetables and Fruits. In: *Food Microbiology: Principles into Practice.* 1st ed. John Wiley and Sons Ltd; 2016; 20: 337-338.

Falagas, M. E., & Karageorgopoulos, D. E. (2009). Extended-spectrum *β*-lactamase-producing organisms. *Journal of Hospital Infection*, *73*(4), 345-354.

Gekenidis, M. T., Rigotti, S., Hummerjohann, J., Walsh, F., & Drissner, D. (2020). Long-term persistence of bla CTX-M-15 in soil and lettuce after introducing extended-spectrum *β*-lactamase (ESBL)-producing *Escherichia coli* via manure or water. *Microorganisms*, *8*(11), 1646.

Government of Northwest Territories, (2022). Nutritional Food Fact Sheet Series [online]. Available at: https://www.hss.gov.nt.ca/en/services/nutritional-food-fact-sheet-series/lettuce#:~:text=Lettuce%20is%20an%20excellent%20source,to%20prevent%20neural%20tube%20defects. [accessed: 22nd February 2023]

Hölzel, C. S., Tetens, J. L., & Schwaiger, K. (2018). Unraveling the role of vegetables in spreading antimicrobial-resistant bacteria: a need for quantitative risk assessment. *Foodborne pathogens*

and disease, 15(11), 671-688.
Kebede, A. A., Bedada, T. L., Teklu, D. S., Beyene, D., & Tullu, K. D. (2022). Occurrence and anti-microbial susceptibility pattern of extended spectrum beta-lactamase producing Enterobacteriaceae in governmental hospitals wastewater in Addis Ababa, Ethiopia. *Tropical Medicine and Health*, *50*(1), 1-16.

NHS, (2023). *Escherichia coli* (*E. coli*) O157 [online]. Available at: https://www.nhsinform.scot/illnesses-and-conditions/infections-and-poisoning/escherichia- coli-e-coli o157 [accessed: 21st February 2023]

Orji JO, Ayogu TE, Nnachi AU, Obaji M, Efunshile AM, Okeh CO, Uzoh CV, Asobie, IM. Bacteriological Quality of Mixed Fruits/Vegetables Salads and Selected Ready-to-Eat Vegetables Sold in Abakaliki Metropolis, Ebonyi State, Nigeria. *World Journal of Pharmaceutical and Life Sciences.* 2017; 3(3): 157-163.

Oyeyipo, F., Adesetan, T. O., Yomi-Bada, Y. W., & Soyemi, L. O. (2021). Screening of Fruit and Vegetable Salads retailed in Ago-Iwoye, Ogun State for Extended-Spectrum Beta- Lactamase Producing Gram-Negative Bacteria. *Equity Journal of Science and Technology, 8*(1): 109 – 115.

Oyeyipo, F., Adesetan, T. O., Yomi-Bada, Y. W., & Soyemi, L. O. (2021). Screening of Fruit and Vegetable Salads retailed in Ago-Iwoye, Ogun State for Extended-Spectrum Beta- Lactamase Producing Gram-Negative Bacteria. *Equity Journal of Science and Technology, 8*(1): 109 – 115.

Phyo, S. S. M., Yu, S. S., & Saing, K. M. (2019). Bacteriological examination of bottled drinking water by MPN method. *Haya: The Saudi Journal of Life Sciences*, *4*(7), 227-232.

Rawat, D., & Nair, D. (2010). Extended-spectrum *β*-lactamases in Gram Negative Bacteria. *Journal of global infectious diseases*, *2*(3), 263.

Romyasamit, C., Sornsenee, P., Chimplee, S., Yuwalaksanakun, S., Wongprot, D., & Saengsuwan, P. (2021). Prevalence and characterization of extended-spectrum *β*- lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from raw vegetables retailed in Southern Thailand. *PeerJ*, *9*, e11787.

Sabino, S., Soares, S., Ramos, F., Moretti, M., Zavascki, A. P., & Rigatto, M. H. (2019). A cohort study of the impact of carbapenem-resistant Enterobacteriaceae infections on mortality of patients presenting with sepsis. *Msphere*, 4(2), e00052-19.

Stoleru, V., Inculet, S. C., Mihalache, G., Cojocaru, A., Teliban, G. C., & Caruso, G. (2020). Yield and nutritional response of greenhouse grown tomato cultivars to sustainable fertilization and irrigation management. *Plants*, *9*(8), 1053.

Toe, E., Dadié, A., Dako, E. & Loukou, G., 2017. Bacteriological quality and risk factors for contamination of raw mixed vegetable salads served in collective catering in Abidjan (Ivory Coast). *Advances in Microbiology*, *7*(06), p.405.

Ülger, T. G., Songur, A. N., Çırak, O., & Çakıroğlu, F. P. (2018). Role of vegetables in human nutrition and disease prevention. *Veg. Importance Qual. Veg. Hum. Health*, 7-32.

Umer, M., Ying, L., Abbasi, B. N., & Riaz, M. M. (2020). SLOW INDUSTRIAL OUTPUT GROWTH EVIDENCE-BASED ON KOHAT ROAD INDUSTRIAL ESTATE PESHAWAR-PAKISTAN. *International Journal of Management & Entrepreneurship Research*, *2*(5), 304-313.

Victoria Frankel, (2022). Raw vs. Cooked - What's the best way to eat your vegetables? [Online]. Available at: https://www.viome.com/blog/raw-vs-cooked-whats-best-way-eat-your- vegetables [Accessed: 16th February 2023]

Zurfluh, K., Nüesch-Inderbinen, M., Morach, M., Zihler Berner, A., Hächler, H., & Stephan, R. (2015). Extended-spectrum-*β*-lactamase-producing Enterobacteriaceae isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. *Applied and environmental microbiology*, *81*(9), 3115-3120.