

The rate of contamination, occurrence, and molecular characterization of *Pseudomonas* isolates in vegetables collected from different regions of sulaimani province-Iraq

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Abstract

Vegetables are a form of delivering pathogenic bacteria, such as *Pseudomonas* spp. This study investigated the rate of vegetable contamination by bacteria with their bacteriological and molecular characterization in Sulaimani Province. The colony forming unit was used to indicate the rate of bacterial contamination per gram of vegetable. As a result, all studied vegetables were found to be highly contaminated by bacteria at high rates, reaching 64,816 bacteria/gm. Among them, coriander showed the highest rate of contamination (89,720 cfu/g), while the carrot had the lowest rate (43,260 cfu/g). The highest rate of bacterial contamination was in Qaladze region and Sulaimani city, while the least contamination was observed in Chamchamal. In total, 54 *Pseudomonas* bacteria were isolated and PCR amplification of 16SrRNA gene (670 bp) was used for the final confirmation. The resistance rate of *Pseudomonas* bacteria was the highest against Amoxicillin, (%98.2) and amoxycilin/clavulanic, (%96.4), while the Ceftriaxone and Ceftazidime remained more affective against the bacteria. Out of 54 *Pseudomonas* isolates, four isolates (7.4%) were found to carry *bla*OXA resistance gene, and two isolates (3.7 %) were positive to *bla*CMY. None of *Pseudomonas* isolates carried *bla*CTX, *bla*SHV or *bla*TEM genes. It is concluded that all vegetable types in the study were contaminated by bacteria at high rates, but the rates were different in the varying types of vegetables. All vegetable samples in all geographical areas were contaminated by *Pseudomonas* bacteria containing isolates that carry *bla*OXA and *bla*CMY genes, which are highly resistant to many antibiotics. Therefore, the vegetables in the studied area are at a threatening level to the public health if not properly handled and treated.

Key Words: Food-borne diseases, Vegetables, *Pseudomonas*, Resistance genes, beta lactamases.

Introduction

Food borne bacteria diseases are problematic and are clinically important. In addition to the pain of illness, it also causes economic losses. One third of people in industrialized countries experience food-borne diseases yearly, reaching six to 81 million cases (Alzoreky and Nakahara, 2003; FAO/WHO 2005, 2005). In the United States, food-borne diseases have affected around 76 million people in one year, including 5,000 deaths (Fan, et al. 2009).

Fresh vegetables and fruits are known as a source of essential dietary nutrients for consumers (Lerici, C. R. Nicoli, 2000), but they can also become a risk of transmitting bacterial diseases (Beuchat, 2002). The number of illness cases are growing with increasing consumption of vegetables and fruits (Pathogens *et al.*, 2014). It has been shown that vegetables have a bigger role in transmitting food borne diseases in comparison to fruits (Garg, N.; Garg, 2010). Many types of pathogenic bacteria, such as *Shigella* spp., *Listeria monocytogenes*, *Salmonella* spp., Shiga toxigenic *E. coli* (STEC), and *Campylobacter* spp., have been found to be associated with food-borne illnesses in ready-to-eat vegetables (European Commission (E.C.), 2002).

The genus of *Pseudomonas* bacteria is the most diverse and common type of bacteria (Franzetti and Scarpellini, 2007; Molina and Pimentel, 2013). Some species of *Pseudomonas* can be found in all countries and in any kind of environment (Jos, J. and In't Veld, 1996; Molina and Pimentel, 2013). In addition, it is also known as the most common psychrotrophic bacteria that causes food spoilage, especially in humid aerobic conditions (Borch, E., Kant-Muermans, M. and Blixt, 1996; Dainty, 1992), and in poultry (Gallo, et al. 1998).

In Iraq, people consume large amounts of varying vegetables daily. Some of the vegetable plants are irrigated with sewage water, so the chance of contamination with pathogenic bacteria is extremely high. Therefore, in this study, an attempt was made to find the rate of vegetable contamination by common bacteria and find the rate of contamination by *Pseudomonas Spp.* in different regions of Sulaimani province. In addition, the antibiotic sensitivity and the rate of beta lactamase resistance gene was detected using PCR.

Material and methods

Sampling

In this survey, the contamination rate of bacteria in vegetables was investigated. Vegetable wash was used to isolate and culture the bacteria and total bacteria count in 54 vegetable samples (10 carrots, 10 leeks, 10 celeries, 10 radishes, and 10 corianders). This was performed in Sulaimany city from October to November 2019. Samples were collected in five regions and towns (14 in Sulaimany, 10 in Chamchamal, 10 in Bazian, 10 in Qaladze, and 10 in Kalar). The samples were collected and put into sterile poly bags under aseptic condition to avoid contamination before transporting to the laboratory. The samples were then processed in the lab by suspending 25 grams of each type of vegetable in 225 ml of normal saline (Anonymous, 2014). The mixture was put into a shaking incubator for 30 minutes at 250 RPM (rotation per minute) and one ml of the wash was taken into an Eppendorf tube for use.

Total bacterial cell counting

Total live bacterial cell count was performed using the colony forming unit method on nutrient agar, MacConkey agar, and Mannitol Salt agar (Neogene Culture Media, Heywood, UK). After inoculation, all the plates were incubated at 37°C for 24 hours, except the bacterial culture Cetrimide agar plate that was used to isolate *pseudomonas* bacteria; this was incubated at room temperature. The colonies were then counted to estimate the number of growing colonies on each plate for every sample.

Isolation of *Pseudomonas* spp.

Bacteriological swabs were used to collect bacterial suspension and to culture them onto MacConkey agar and Cetrimide agar. After 18-24 hours of incubation at 37 °C, pink colonies on the MacConkey agar were chosen for further confirmation by culturing the bacterial suspension onto Cetrimide medium. *Pseudomonas* made a large mucoid green colony on the Cetrimide agar (Taylor *et al.*, 1977). Biochemical tests were carried out for identification and confirmation of *pseudomonas* species using oxidase and catalase tests. The final confirmation was made by PCR amplification of 12SrRNA using primers specific to *Pseudomonas* spp. and to *Pseudomonas aurogenosa*.

Antimicrobial Susceptibility Testing

Susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller Hinton agar. 6 different types of antibiotics were used, including ceftriaxone (CRO30µg), ceftazidime (CAZ30µm), cefotaxime (CTX30µm), amoxycilin/clavulanic acid 2/1 (AMC), meropenem (Mem10µm), and amoxicillin (AX25µm) (Bioanalyse, Ankara, Turkey).

Polymerase Chain Reaction (PCR)

DNA of bacteria was extracted by the boiling method at 99 °C in 100 microliters of sterile distilled water for 10 minutes (Dashti *et al.*, 2009). To confirm *pseudoscience* species, a set of primers were used. Another set of specific primers were used to confirm *pseudomonas aeruginosa* (El-roos *et al.*, 2013; Mohammed *et al.*, 2015). Primers that were used to detect five beta lactamase resistance genes (blaCTX, CMY, SHV, TEM, OXA) were also used to detect the existence of resistance genes in *pseudomonas* species (Ahmed *et al.*, 2007, 2009).

PCR was performed in a 20-microliter reaction mixture using an applied bio system thermocycler. The mixture was composed of 10µL of 2X premix Red Taq polymerase, 2µL DNA, 1µL H₂O, and 2µL primers. The PCR condition started at 95 °C for 5 min, and then 35 cycles of 95 °C for 30 sec, 55 °C for 30 sec, and 72 °C for 30 sec, with a final extension temperature of 72 °C for 7 min. 10µL of the PCR mixture was run on 1% agarose gel using DNA gel electrophoresis system at 100 V for 30 min. Then the images of DNA were taken using smart Doc gel imaging system.

Results

The rate of bacterial contamination in vegetables

To know the rate of bacterial contamination among different kinds of vegetables in different locations in Sulaimani Province, varying kinds of vegetable samples were aseptically collected for laboratory processing and bacteriological studies. Five types of vegetables (leek, carrot, celery, radish, and coriander) were collected in five different locations (Sulaimany, Chamchamal, Bazian, Kalar and Qaladze).

Total bacterial counting:

Total bacterial counting on nutrient agar

Since nutrient agar is a non-selective medium and all types of bacteria can grow on it, the number of bacteria on this agar was counted to detect the total bacterial contamination in vegetables. From 50 samples of different vegetables, the rate of contamination by all types of bacteria was very high, (64,816 bacteria/gm). Coriander recorded the highest number of contamination, (89,720 cfu/g), while the carrot had the lowest rate of contamination (43,260 cfu/g). The rate of contamination in leek, celery and radish were 77,060 cfu/g, 58,920 cfu/g, and 55,120 cfu/g, respectively (Figure 1A). The number of bacteria (cfu) on nutrient agar was also counted according to different regions. Qaladze had the highest rate of contamination (93,040 cfu/g), while Kalar had the lowest rate (40,200 cfu/g) (Figure 1B).

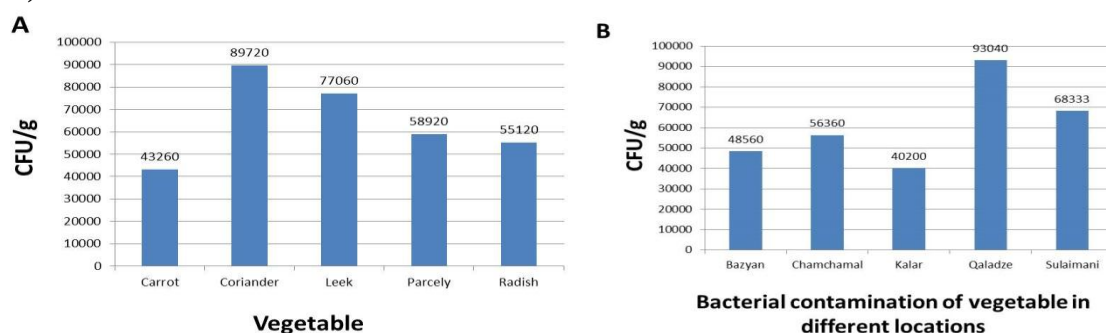


Figure 1. The rate of total bacterial contamination in vegetables.

A. The rate of contamination according to different types of vegetables.

B. Bacterial contamination rate of vegetables according to different geographical areas.

Total bacterial counting on MacConkey agar

In this study, MacConkey agar was used to indicate the rate of Enterobacteriaceae contamination in vegetables, which may source from fecal contamination. This may cause fecal oral transmission of pathogenic bacteria (Warburton, et al. 1994). The rate of enteric bacteria contamination in vegetables was very high, (26,044 bacteria/gm). The highest rate was recorded in celery (39,640 cfu/g), while the lowest rate was detected in radish (10,880 cfu/g). The rate of contamination in leek, coriander and carrot were 25,960 cfu/g, 27,780 cfu/g, and 25,960 cfu/g, respectively (Figure 3 2A). In addition, the numbers of bacteria on MacConkey agar among different locations was counted. Qaladze had the highest rate of contamination of Enterobacteriaceae (67,600 cfu/g), while Kalar had the lowest rate (3,120 cfu/g) (Figure 2B).

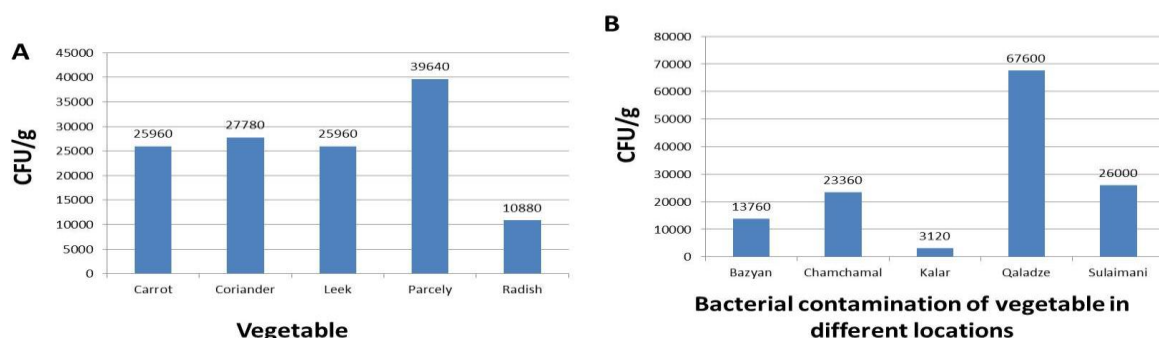


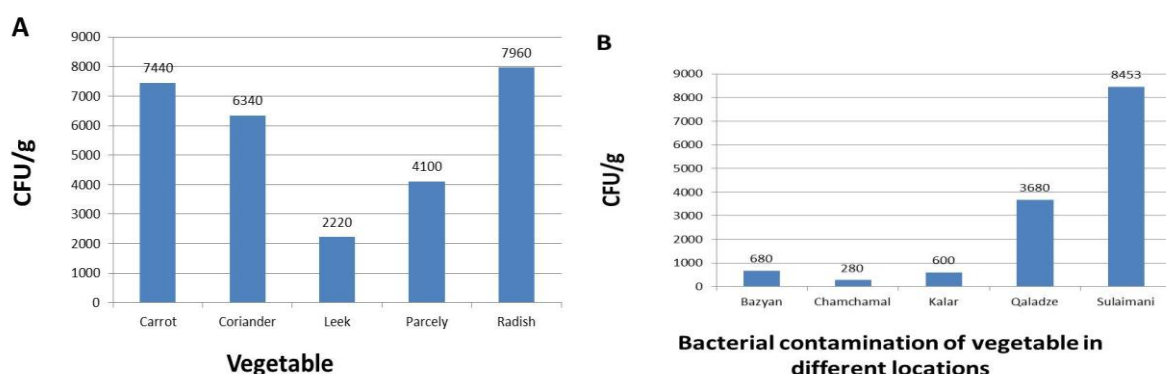
Figure 2. The rate of total bacterial contamination (Enterobacteriaceae) in vegetables.

A. The rate of bacterial (Enterobacteriaceae) contamination in different vegetables.

B. Bacterial contamination rate (Enterobacteriaceae) of vegetables according to different geographical areas.

Total bacterial counting on Mannitol Salt agar

This media was used to indicate the rate of contamination of bacteria like *Staphylococcus* species that can grow on high salt containing medias, such as Mannitol Salt agar. The total rate of *Staphylococcus* contamination in all types of vegetables was high; (5,612 bacteria/gm). Radish had the highest rate of contamination (7,960 cfu/g), while leek had the lowest rate (2,220 cfu/g). The rate of contamination among carrot, coriander and celery were 7,440 cfu/g, 6,340 cfu/g, and 4,100 cfu/g, respectively (Figure 3A). The same study was carried out according to different geographical places. Sulaimani had the highest rate of contamination (8,453 cfu/g), while Chamchamal had lowest rate (280 cfu/g) (Figure 3B).



Isolation of *pseudomonas* bacteria

There are many sources of transmitting pathogenic bacteria to humans, one of them being vegetables (Beuchat, 2002; Pathogens *et al.*, 2014). *Pseudomonas* bacteria were isolated on selective and differential media. Three methods were used to isolate and identify *pseudomonas* bacteria. After isolation of bacteria on MacConkey agar, the suspected colonies were further differentiated by culturing them on a selective and differential media, Cetrimide agar. *Pseudomonas* bacteria produce a green, fluorescent color which is a unique characteristic of *Pseudomonas* on Cetrimide agar. Positively selected colonies were then further confirmed by biochemical tests, oxidase and catalase. *Pseudomonas* bacteria is one of the five clinically important gram negative bacteria, which is positive to oxidase test. Therefore, this test was used to confirm *pseudomonas* bacteria. The last confirmation was done specifically by PCR amplification of a gene using primers specific only to *Pseudomonas* bacteria. In this study, 54 *Pseudomonas* bacteria were isolated, and for this selection, an eye observation was used to look for the development of a bright fluorescent colored colony on Cetrimide and Mueller Hinton agar media (Taylor *et al.*, 1977).

16SrRNA gene (670 bp) primers specific for *pseudomonas* species was used for further confirmation of *pseudomonas* species (Mohammed *et al.*, 2015). All of the isolated bacteria were positive for this gene (Figure 4). 16SrRNA gene specific for *pseudomonas aeruginosa* was then used (El-roos *et al.*, 2013; Mohammed *et al.*, 2015), but all of the samples were negative for this gene. Fortunately, there were no pathogenic *pseudomonas aeruginosa* bacteria in the vegetable samples that may threaten human health.

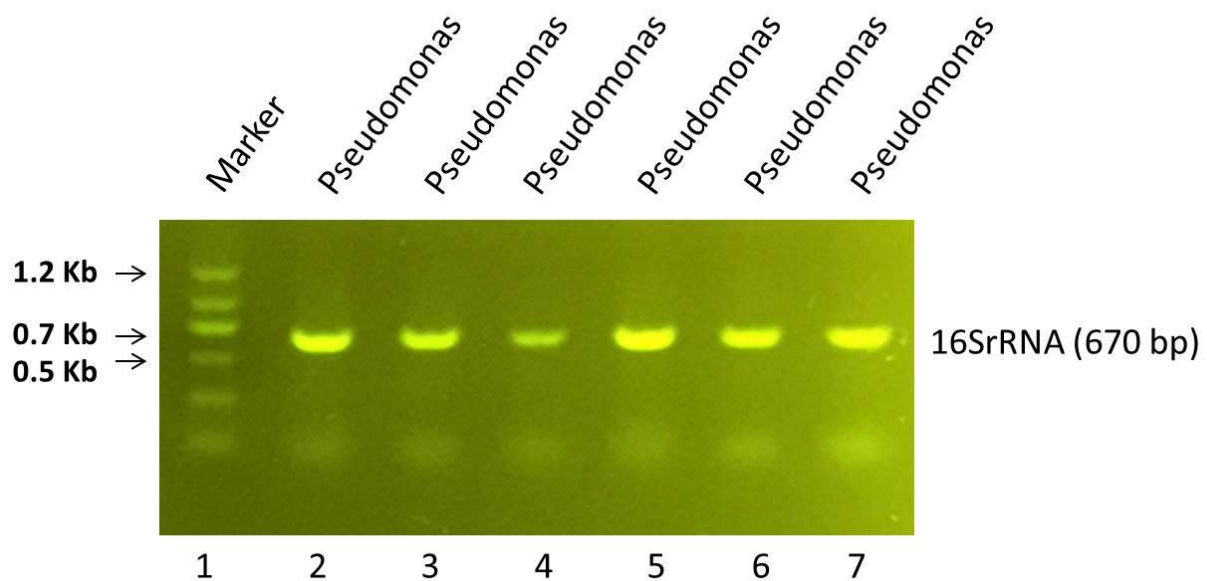


Figure 4: PCR confirmation of *Pseudomonas* species. 16SrRNA gene (670 bp) specific for pseudomonas species were amplified and run on 1% DNA agarose gel using 1.2 Kb DNA ladder.

Antibiotic sensitivity testing

An antibiotic sensitivity (or susceptibility) test was done to indicate the resistant rate of *pseudomonas aeruginosa* bacteria against different antibiotics. The test was performed using the Kirby-Bauer disk diffusion method on Mueller Hinton agar. Six different types of beta lactam antibiotics were used, including ceftriaxone(30 μ g), ceftazidime(30 μ m), cefotaxime(30 μ m), amoxicillin/clavulan acid2/1, meropenem(10 μ m), and amoxicillin(25 μ m) (bio Maxima S.A). In this test, the rate of resistance to different antibiotics in *Pseudomonas* was detected. Among 54 samples of *pseudomonas* bacteria, the percentage rate of resistance for antibiotics was as follows: (CRO30/16.36%), (CAZ30/16.36%), (CTX30/52.72%), (AMC3/96.4%), (MEM10/25.5%) and (AX25/98.2%)-(Figure 5).

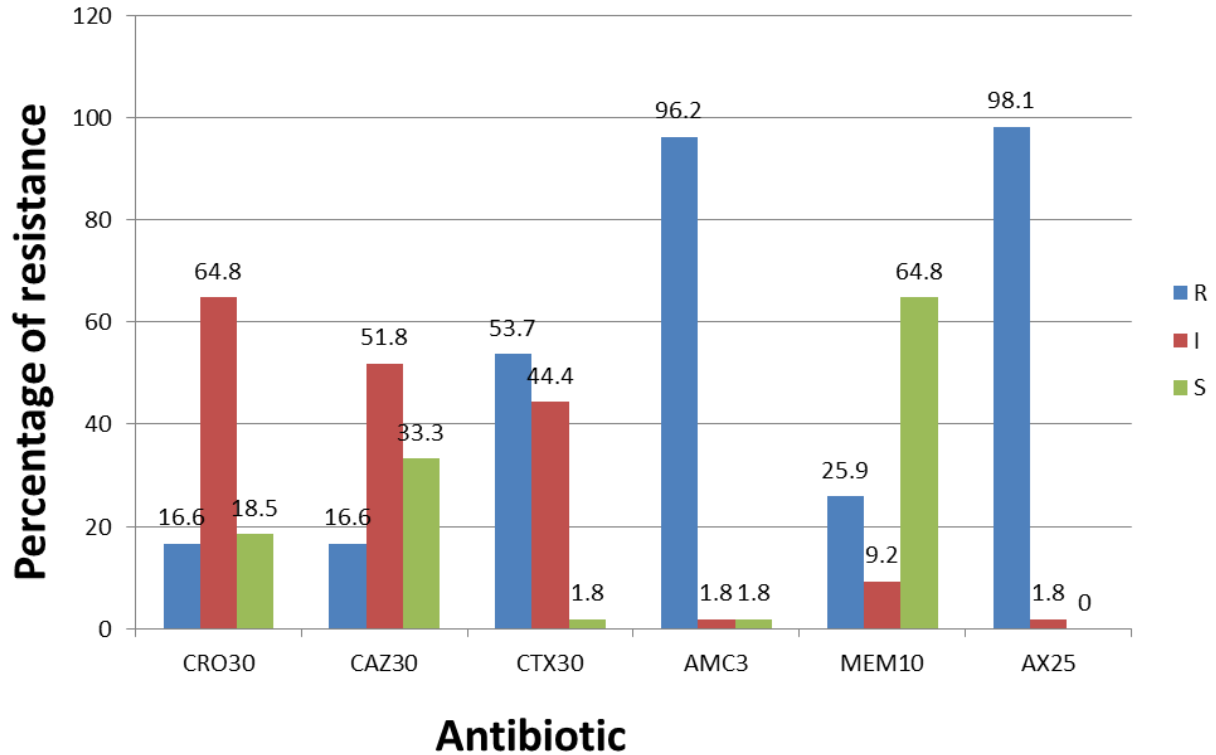


Figure 5. The rate of antibiotic resistance in *Pseudomonas* isolates. The rate of antibiotic resistance was indicated in 54 *Pseudomonas* isolates recovered from different types of vegetable.

Table1. Drug resistance scores of *Pseudomonas* isolated from vegetable samples.

Samples	CRO30	CAZ30	CTX30	AMC3	MEM10	AX25
P1	I(17)	R(14)	R(14)	R(0)	S(17)	R(0)
P2	I(18)	I(15)	R(13)	R(0)	S(17)	R(0)
P3	I(18)	I(17)	I(15)	R(0)	S(20)	R(0)
P4	R(12)	R(12)	R(0)	R(0)	S(28)	R(0)
P5	R(10)	R(12)	R(0)	R(0)	I(15)	R(0)
P6	S(21)	I(17)	R(12)	R(0)	S(18)	R(0)
P7	I(18)	S(20)	R(14)	R(0)	S(20)	R(0)
P8	I(17)	S(18)	I(16)	R(0)	S(18)	R(0)
P9	I(18)	S(18)	I(15)	R(0)	S(17)	R(0)
P10	I(18)	S(19)	I(16)	R(0)	S(18)	R(0)
P11	I(15)	I(16)	R(11)	R(0)	S(16)	R(0)
P12	I(18)	I(12)	I(15)	R(0)	S(19)	R(0)
P13	S(22)	S(20)	I(17)	R(0)	S(20)	R(0)
P14	I(18)	I(17)	I(15)	R(0)	S(18)	R(0)
P15	R(13)	S(18)	R(12)	R(0)	S(33)	R(0)
P16	R(0)	I(17)	R(0)	R(0)	I(15)	R(0)
P17	S(26)	S(27)	I(20)	S(18)	S(45)	R(0)
P18	I(17)	I(17)	R(14)	R(0)	R(10)	R(0)
P19	I(14)	I(17)	I(15)	R(0)	S(17)	R(0)
P20	I(17)	I(17)	R(11)	R(0)	I(15)	R(0)
P21	S(21)	S(20)	I(18)	R(0)	S(20)	R(0)

P22	I(18)	S(18)	I(15)	R(0)	S(20)	R(0)
P23	I(17)	I(17)	I(16)	R(0)	S(20)	R(0)
P24	R(12)	S(20)	R(0)	R(0)	S(22)	R(0)
P25	R(10)	I(15)	R(0)	R(0)	S(17)	R(0)
P26	I(16)	S(21)	R(13)	R(0)	S(33)	R(0)
P27	I(18)	I(17)	R(13)	R(0)	S(30)	R(0)
P27-A	S(21)	I(17)	R(12)	R(0)	S(22)	R(0)
P28	I(20)	S(21)	I(15)	R(0)	S(26)	R(0)
P29	I(17)	S(18)	I(15)	R(0)	S(20)	R(0)
P30	I(15)	R(13)	R(12)	R(0)	R(0)	R(0)
P31	I(17)	R(14)	R(13)	R(0)	R(0)	R(0)
P32	I(16)	R(13)	R(11)	R(0)	R(0)	R(0)
P33	I(16)	R(11)	R(0)	R(0)	S(24)	R(0)
P34	I(14)	R(14)	R(12)	R(0)	R(0)	R(0)
P35	I(14)	I(15)	R(12)	R(0)	I(15)	R(0)
P37	I(17)	I(16)	R(12)	R(0)	S(19)	R(0)
P38	R(13)	I(17)	R(0)	R(0)	R(0)	R(0)
P39	S(21)	I(17)	R(13)	R(0)	R(10)	R(0)
P40	I(16)	I(17)	R(12)	R(0)	S(17)	R(0)
P41	R(12)	I(17)	R(14)	R(0)	I(15)	R(0)
P42	I(15)	I(15)	R(10)	R(0)	R(0)	R(0)
P43	I(16)	I(17)	I(15)	R(0)	R(0)	R(0)
P44	I(17)	I(17)	I(15)	R(0)	R(10)	R(0)
P45	I(20)	I(17)	I(15)	R(0)	R(11)	R(0)
P46	I(16)	I(15)	I(17)	R(0)	R(10)	R(0)
P47	I(16)	S(21)	R(12)	R(0)	S(17)	R(0)
P48	R(13)	I(15)	I(15)	R(0)	S(18)	R(0)
P49	I(20)	R(13)	I(15)	R(0)	R(10)	R(0)
P50	S(30)	S(24)	I(15)	R(0)	S(25)	R(0)
PS21	S(22)	S(23)	I(20)	I(17)	S(32)	I(14)
PS22	S(26)	S(22)	S(28)	R(0)	S(26)	R(0)
PS23	S(24)	S(20)	I(20)	R(0)	S(26)	R(0)
PS24	I(17)	S(20)	I(15)	R(10)	R(13)	R(0)

Resistance (R) means the antibiotic lost its ability to inhibit the growth of bacteria, intermediate (I) means that the antibiotic works on bacterial growth moderately, and sensitive (S) means the antibiotic has the ability to inhibit the growth of bacteria. The scores are diameters of the inhibition zone in millimeters.

Drug resistance genes

This study investigated the existence of β -lactamase resistance genes (CMY, OXA, CTX, SHV, TEM) in *Pseudomonas* bacteria by PCR amplification of their conservative region using gene specific primers (Ahmed *et al.*, 2007, 2009). All *Pseudomonas* isolates showed negative results to three types of β -lactamase resistance genes, blaCTX, blaSHV and blaTEM. This means none of the isolates carry these types of resistance genes on their chromosome and plasmids. Out of 54 *pseudomonas* isolates, four isolates carried blaOXA gene (7.4%), and two isolates were found to harbor blaCMY resistance gene (3.7 %) (Figure 6).

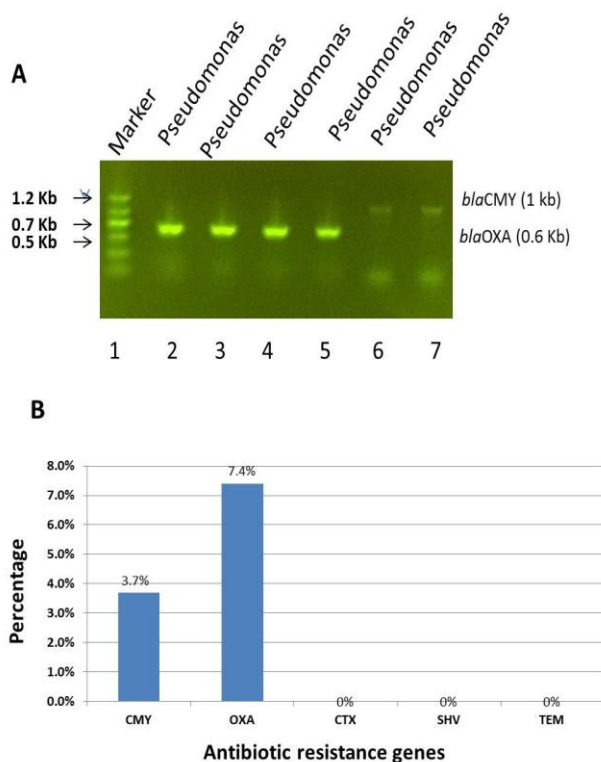


Figure 6: The rate of antibiotic resistance genes in *Pseudomonas* species.

A. Amplification of b-lactam resistance genes in *Pseudomonas*. *blaOXA* was recovered in four isolates (Lane 2-5) and the two genes of *blaCMY* were recovered in two isolates (Lane 6 and 7).

B. The rate of antibiotic resistance genes in *Pseudomonas* spp. isolated in the study area.

Discussion

Food poisoning syndrome ~~resulted~~ results from ingestion of a wide ~~variety~~ variety of food and vegetables contaminated with pathogenic organisms such as {bacteria, virus, parasite, and fungi}, and their toxins and chemicals. Vegetables become contaminated by bacteria in fields during growing due to the use of organic fertilizer and contaminated irrigation water, or during harvesting, processing, distribution, sell and consumption (Beuchat, 2002; European Commission (E.C.), 2002; Garg, N.; Garg, 2010; Lerici, C. R. Nicoli, 2000).

Bacterial contamination rates are different according to the different type of vegetable and place (Garg, N.; Garg, 2010). For example, carrots are cleaner than radishes because of its shape which cannot carry large amounts of dirt. It is also easierto clean and wash. Celery was found to be contaminated more than the other types of vegetables because celery is usually dirtier than carrot. This may be due to the fact that celery grows on the ground and is more exposed to environmental contamination. It also has a shape that is able to collect more bacteria and dust in its leaves compared to the other types of vegetables, including carrot and radish, which are smoother. In conclusion, all types of vegetables can become contaminated by different ratios, so proper washing of vegetables is necessary before consumption.

The total bacteria count in this study revealed that the contamination of vegetables by different types of bacteria is very high in the area. The average number of bacteria found in all the vegetables (50 samples) was 64,816 bacteria/gm. Coriander recorded the highest number of contamination, (89,720 cfu/g), while the carrot had the lowest rate of contamination (43,260 cfu/g) (Figure 1). This is an indication that the vegetables in the area are not safe for consuming directly from the field, but needs proper washing and disinfecting to avoid catching diseases. Contamination of vegetables by the enteric bacteria is much higher than *Staphylococcus* species. The rate of contamination of *Enterobacteriaceae* group of bacteria, including *Pseudomonas*, was much higher (reaching 26,044 bacteria/gm CFU/gm in average), than *Staphylococcus* (reaching 5,612 CFU/gm in average) (Figures 2 and 3). This is an indication that vegetables are mostly contaminated by fecal bacteria in the area of study because farmers use sewage water for watering and growing of vegetables. Presence of *pseudomonas* in the

soil is another reason. Sewage water is a carrier of large numbers of enteric bacteria, and it usually mixes with stream and river water in the area. Large areas of agriculture fields depend on these sources of water for irrigation. Therefore, the plants and vegetables become contaminated and carry huge numbers of bacteria, especially enteric bacteria. Consequently, they become a source of food borne diseases if not properly washed and cleaned before use. The previous finding agrees with the results because they confirmed that *Pseudomonas* species is the most common bacteria causing contamination in vegetables (Raposo *et al.*, 2017).

The rate of total bacteria and *pseudomonas* in vegetables vary in different regions in the studied area. For example, vegetables in Qaladze are dirtier and more contaminated than those in Kalar. This can depend on the freshness of water that is used for irrigation, or the farmer's responsibility by taking care of the vegetables during processing and transportation. Irrigation by sewage water is common especially around big cities, which causes heavier contamination of vegetables in some areas such as Sulaimani, and Qaladze. In contrast, Chamchamal uses well water (clean water) for irrigation, which is the main reason for having the lowest rate of bacterial contamination than the other areas. So the quality and sanitation of water is a key point to indicate the rate of contamination in vegetables.

The contamination of vegetables and fruits is related to many factors, starting from irrigation, harvesting, handling, processing, etc. (Kader, A. and Barrett, 1996). The rate of *Pseudomonas* species was relatively high in this study, but the pathogenic species, *Pseudomonas aurogenosa*, was not recorded. All vegetable samples in this study contained *Pseudomonas* species bacteria, 100%. The finding agrees with the previous conclusions that *Pseudomonas* bacteria is one of the common food spoilage bacteria in vegetables and fruits (Rahman, 2002; Raposo *et al.*, 2017).

Antibiotic resistance occurrence was indicated for all *Pseudomonas* isolates recovered in this study. Therefore, by using antibiotic sensitivity testing, it was realized that most of the isolated bacteria became resistant for many antibiotics in different ratios. The highest resistance was to amoxicillin and amoxycilin/clavulanic acid, which reached AX25 %98.2 and AMC3 %96.4, respectively (Figure 5). These resistance bacteria may be sourced from fecal contamination of sewage water or maybe are natural environmental bacteria, but nevertheless have developed resistance against many antibiotics. These resistant bacteria are problematic if passed into the population because they become a source of infection and treatment of patients will be impeded due to the ability of resistance bacteria to reject antibiotics during treatment.

Most of the reasons of bacterial resistance are due to having drug resistance genes. When these beta lactamase genes exist, it enables the bacteria to produce special proteins to degrade antibiotics (Grover *et al.*, 2013; Khanna *et al.*, 2012; Vaidya, 2011; Zhang *et al.*, 2012). In this study, many *Pseudomonas* bacteria were found harboring bla-CTX and bla-OXA genes, which may help bacterial survival during antibiotic treatment (Figure 6). The existence of resistance genes in bacteria may come from bacteria of animal and human sources, rather than from soil, water and vegetables because natural bacteria of the environment carry resistance genes less often (Alaa *et al.*, 2020).

Conclusion

The rate of bacterial contamination of vegetables is very high, reaching 64,816 bacteria per gram of vegetable. The number of contamination by enteric bacteria, (39640/gm) is more than *Staphylococcus* species, (5,612 bacteria/gm). Total bacterial count revealed that the samples of Sulaimani town and Qaladze had the highest range of contamination due to using stream and river water mixed with sewage water for irrigation. The rate of vegetable contamination by *Pseudomonas Spp.* was 100% and it was mostly found on celery and radish at a high range. The rate of resistance of *Pseudomonas* is very high against many types of antibiotics, AX25 (%98.2) and AMC3 (%96.4). In addition, two types of beta lactamase resistance genes, *blaOXA* and *blaCMY*, were recovered in *Pseudomonas*. Finding a large number of resistant bacteria in vegetables shows that the vegetables are not safe and are a threat to public health because the treatment will be difficult when people become infected with these bacteria through consuming contaminated vegetables.

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