

Research Article

The study to evaluate the prevalence of *Yersinia* species in salad sold and water supplied in Quetta city, Pakistan

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Abstract

The spectrum of foodborne illness has been changed dramatically because of modern life style. This pilot study was intended to investigate the contaminated salad and water with *Yersinia* species and to determine their antimicrobial susceptibility pattern in 200 collected samples. Typical biochemical tests along with advanced molecular techniques were adopted for characterization of the isolated bacteria. In salad sample 20% (n=100) were contaminated with *Yersinia* species when biochemical tests were adopted for identification, however polymerase chain reaction (PCR) test confirmed the contamination of 27% of the samples. Out of 100 water samples analyzed 6 and 8 were found contaminated as confirmed by biochemical and PCR respectively. PCR based species typing was performed on the *Yersinia* isolated from the analyzed samples. The PCR confirmed isolates were further investigated by multiplex PCR to identify the specific species involved. Multiplex PCR demonstrated that 34 were *Yersinia enterocolitica* and 5 were identified as *Yersinia pseudotuberculosis*. Further segregation of multiplex PCR outcomes demonstrated that salad samples were most contaminated with *Y. enterocolitica* (n=27) and only two water samples were found positive. Similarly, out of five *Y. pseudotuberculosis* isolated, 4 were detected in salad samples while only 1 water sample was found contaminated with *Y. pseudotuberculosis*. Importantly, no sample was found contaminated with *Y. pestis*. The isolated *Yersinia* species were susceptible to Gentamicin, Tobramycin, Chloramphenicol, Nalidixic acid, Levofloxacin, Ciprolopxicin, Imipenum, Ceftaxine, and Cefrixone but resistant against Ampicillin, Amoxicillin, Tetracycline and Fosfomycin.

Keywords: Antibiotic sensitivity; Salad; Quetta; Water; *Yersinia* contamination

Introduction

Foodborne illnesses are widespread and pose a great health concerns globally [1, 2] and among these diseases yersiniosis is

among the top three frequently occurring infections in European Union (EU). Yersiniosis is caused by different *Yersinia* species and is documented as zoonotic

gastrointestinal problem after campylobacteriosis and salmonellosis [3]. Although, yersiniosis is disease of temperate zones, but it's prevalence has been reported around the globe. During 2007, the outbreaks of yersiniosis ranked it at third among the zoonotic diseases in EU [4].

Yersinia is member of *Enterobacteriaceae* family and this genus is considered as pathogenic because of their diversity and nature to cause several diseases. Genus *Yersinia* has 17 different species and only three named as *Y. pseudotuberculosis*, *Y. pestis* and *Y. enterocolitica* have been mostly involved in human diseases [5]. The remaining species are considered as non-virulent but in certain cases may lead to mild infections [6].

Foodborne diseases are mainly caused by *Y. enterocolitica* and mostly it can be zoonotic in nature [7]. In addition to foodborne illness, *Y. enterocolitica* also cause waterborne diseases when contaminated water has been consumed [8]. *Y. pseudotuberculosis* is less prevalent [9] as compared to *Y. enterocolitica* although has great potential to cause severe sickness [10]. Consumption of contaminated vegetables [11] and water [12] with *Yersinia* are the main cause of illness [13].

The epidemiology of infections and diseases caused by *Yersinia spp.* is complex as the cases are mostly sporadic and route cause of the disease is difficult to establish [14]. However, several outbreaks of *Yersinia* around the globe have been documented [15].

This pilot study was designed to assess the contamination of salad and water with *Yersinia* in Quetta city.

Materials and methods

Sample collection

A total of 200 samples were analyzed (100 ready-to-eat salads and 100 samples of household tap water) for the detection of *Yersinia* species. For this purpose the salad

samples were purchased from different vegetables market of Quetta and water samples were obtained from different locations of Quetta city. The salad samples were instantly stored in sterilized stomacher bags and sterilized bottles were used to collect the water samples. After collection, the samples were transported to Bacteriology Laboratory, Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan in ice containing box. All samples were processed within 4-5 hour.

Enrichment of *Yersinia*

The United States Food and Drug Administration (FDA) method was adopted for isolation of *Yersinia* species. Briefly a 25g of food and 25ml of water sample were aseptically inoculated in 225ml of peptone sorbitol bile broth (PSBB) with the ratio of 1:10 and the samples were homogenized and incubated in anaerobic conditions for 24 hours at 37°C. After incubation 0.5ml of homogenized aliquots were streaked on differential/selective media (Cefsulodin-Irgasan-Novobiocin Agar; CIN, McConkey agar). These culture plates were incubated (18-24 h at 37°C) to have the bacterial growth. The suspected colonies were subjected to Gram staining and microscopy followed by different biochemical tests and finally the PCR.

DNA extraction and PCR

The boiling method was used to extract the DNA from the biochemically identified isolates [16].

Different primers targeting the conserved genes of *Y. pseudotuberculosis*, *Y. pestis* and *Y. enterocolitica* were used for multiplex PCR as has been reported earlier [16-19]. Standard protocol for polymerase chain reaction was adopted as has been described previously [20]. After amplification the samples were subjected to electrophoresis and were visualized and documented.

Antibiotic sensitivity test

PCR confirmed *Yersinia* isolates were subjected to 14 different antibiotics sensitivity test as described by EUCAST (FAD) 2015. Commercially available antibiotics discs Amikacin (30µg), Amoxicillin-clavulanic acid (30µg), Ampicillin (10µg), Cefotaxime (30µg), Cefrixone (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Gentamycin (10µg), Nalidixic acid (30µg), Tetracycline (30µg), Tobramycine (10µg), Levofloxacin (5ug), Fosfomycin (10ug), and Imipenem (50ug) were used. An inhibition zone produced by respective drug was measured and scoring as sensitive, intermediate and resistant was made as per standards of EUCAST.

Results and discussion

The results of this study revealed that 35 samples out of 200 analyzed were positive for *Yersinia* species when PCR was employed and as expected PCR assay was proved more sensitive to biochemical tests as these tests were only able to detect the contamination in 26 samples as shown in (Table 1). Moreover, salad samples were more affected compared to water samples (Table 1).

Specific primers used to detect the involvement of different *spp* demonstrated that *Y. enterocolitica* was the most contaminant of salad and water samples followed by *Y. pseudotuberculosis* as shown in (Table 2).

Yersinia species are major cause of human disease, nevertheless, this bacterium is present everywhere in natural environment, and can be isolated from soil, water and food. In our findings, the overall the detection of *Yersinia* species was 35 (85%) and 5 (14.28%) for *Y. enterocolitica* and *Y. pseudotuberculosis* respectively. These finding are almost similar to the previous studies conducted in United Kingdom [21, 22]. In contrast to our findings the

prevalence of *Y. enterocolitica* [23-25]. Darbs *et al.*, (1985) reported lower contamination of pathogens in salad and vegetable compared to our findings. However some attribute that can play an important role in increase or decrease of incidence of the *Yersinia enterocolitica* are method of isolation, geographical regions and seasonal change. To keep the vegetables fresh, use of water might be the source of contamination [26]. Similarly, during production, packing, harvesting and transportation of fresh fruits and vegetables can contract microbes [26]. In our result the incidence of *Y. enterocolitica* in water are similar to the findings of the studies conducted in India [26, 27]. Many researchers around the world reported the higher prevalence of *Y. enterocolitica* in water compared to our findings [28 - 30]. Lower prevalence of *Y. enterocolitica* was also reported worldwide by many researchers [31, 32]. *Y. enterocolitica* had been reported as the most prevalent species in contamination of drinking water which could be because of faulty water supply system. It may also occur because of sewage over flow from sewer collection system and may contaminate the water supply system [33].

In this study we were also interested to check the antibiotic susceptibility of the isolated *Yersinia* spp. In our findings all the *Yersinia* isolates were susceptible to Gentamicin, Tobramycin, Chloramphenicol, Nalidixic acid, Levofloxacin, Ciprofloxacin, Imipenem, Ceftaxime, and Cefrixone but show resistance against Ampicillin, Amoxicillin, Tetracycline and Fosfomycin as shown in (Table 3).

Y. enterocolitica has been reported sensitive when Gentamicin, Chloramphenicol and Ciprofloxacin are used and our results are in agreement with those reports [34]. Similarly, it has been also reported that *Yersinia* showed resistant to amoxicillin [35].

Resistance of *Y. enterocolitica* against Tetracycline was reported by [36] which are in accordance with the present study. It has been reported that isolates of *Y. enterocolitica* were resistant to Ampicillin, Nalidixic acid and Ciprofloxacin [30]. Several studies have reported the resistance of Tetracycline, as observed in this study [37-39]. Similarly, resistant against

Tetracycline has been documented previously [35, 40].

Our findings showed that most active pharmacologic agents were Gentamicin, Chloramphenicol, and Trimethoprim. Gentamicin, Chloramphenicol and Ciprofloxacin could be used as drug of choice against infections of *Yersinia*.

Table 1. Prevalence of *Yersinia* in ready to eat salad sample and drinking water sample through conventional and PCR methods

No of samples	Salad		Water		
	Conventional method	PCR method	No of sample	Conventional method	PCR method
100	20 (20%)	27 (27%)	100	6 (6%)	8 (8%)

Table 2. Prevalence of PCR confirmed isolates

<i>Yersinia</i> spp.	Salad	Water
<i>Y. enterocolitica</i>	27	6
<i>Y. pseudotuberculosis</i>	4	2

Table 3. Antibiotic susceptibility test results of *Yersinia* isolates

Antibiotic	POTENCY	Susceptibility
Amikacin	30	Intermediate
Gentamicin	10	Sensitive
Tobramycin	10	Sensitive
Ampicillin	10	Resistance
Amoxicillin	10	Resistance
Chloramphenicol	30	Sensitive
Tetracycline	30	Resistance
Nalidixic acid	30	Sensitive
Levofloxacin	5	Sensitive
Ciprofloxacin	10	Sensitive
Imipenem	5	Sensitive
Fosfomycin	50	Resistance
Ceftaxine	30	Sensitive
Cefrixone	30	Sensitive

Conclusion

The study concluded that the salad could serve as a source of infection and the water supplied to different areas of Quetta city is contaminated with pathogenic strains of

Yersinia. The plausible explanation to salad contamination could be the use of contaminated water for irrigation or later sprinkling to keep them fresh. The water contamination may be because of

leakage/breakage in water supply system, which may serve as an inlet for sewage water in the supply line.

Authors' contributions

Conceived and designed the experiments: H Mengal, A Samad & MT Asmat, Performed the experiments: H Mengal & MZ Mustafa, Analyzed the data: F Abbas, N Sajjad, H Ishtiaq, H Rahim & D Ghilzai, Wrote the paper: All authors contributed to write the manuscript.

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