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Short Communication

Prevalence of Foodborne Pathogens in Food Items in Quetta, Pakistan

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ABSTRACT

Foodborne pathogens pose a great health risk globally. This study was conducted to examine the prevalence of deadly food contaminants like *Salmonella* spp., *Listeria monocytogenes, Campylobacter jejuni* and *E. coli* (O157:H7) in various types of food items consumed locally. Total of 800 most common food samples such as chicken, beef meat, raw milk, vegetables and salad samples were collected from retail market, the overall contamination of food samples with infectious agents was 48.37%. *Campylobacter jejuni* was recorded much higher (28.99%) compared to other foodborne pathogens. Food samples were also found positive for *Salmonella* spp. (19%), *E. coli* (O157:H7) (8%) and 1.25% for *Listeria monocytogenes*. 5% tested food samples were found co-infected with at least two pathogens. The results urge to adopt proper food hygiene practices to reduce the incidence of food-borne diseases.

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Authors' Contributions
AS, TMA and FA designed the study. AS collected the samples. AS and TMA performed the experiments. ZA and OP analyzed the data. AS and TMA wrote the paper.

Key words
Foodborne pathogens, PCR, *E. coli* (O157:H7), *Campylobacter jejuni*, *Salmonella*, *Listeria monocytogenes*.

Foodborne diseases represent a worldwide health problem caused by opportunistic pathogens mingled in food and water. Main harbor of foodborne pathogens are poultry/chicken products such as meat sold at retail markets, undercooked meat, raw milk, vegetables and fruits (Tahir *et al*., 2017; Zaulet *et al*., 2016; Denis *et al*., 2016; Raufu *et al*., 2014). In 2005, WHO reported that 1.8 million individuals worldwide died because of diarrheal caused by foodborne pathogens (Hird *et al*., 2009). The global risk of foodborne pathogens has been aggravated by virtue of trade globalization and the affluence of travel around the world (Hird *et al*., 2009; Weama *et al*., 2016). The source of contamination might be the animal, the environment or uncleanness during food processing. Another cause may be the animal feces which may serve as a source of contamination during slaughtering. Foodborne pathogens are considered as the major sources of zoonotic infections and play important role in mortality and morbidity in developing countries and billions of dollars are spent to treat these infections. *Salmonella* sp., *Campylobacter jejuni*, *Clostridium botulinum, Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica* (Abdallah et al., 2013; El-Zamkan and Hameed, 2016) and *Salmonella* (Denis *et al*., 2016; Mandal *et al*., 2011) are considered to be the main culprits in foodborne infections.

Besides traditional culture techniques used for the detection of food borne pathogens in food DNA based approaches have also been adopted for more efficient and reliable identification of pathogens (Prasad *et al*., 2009).

The aim of the present study was to explore the prevalence of important foodborne bacterial pathogens in various foods consumed locally by employing modern and conventional methods.

Materials and methods

This study was carried out in Quetta, Pakistan from February 2016 to December 2016. A total of 800 samples were collected randomly from different areas of Quetta city. Out of 800 samples, 200 chicken meat samples (100 raw fresh chicken + 100 processed), 200 beef meat samples (100 diced +100 minced), 200 raw milk samples (100 from shop + 100 dairy farm), 100 vegetables samples and 100 salad samples were collected and preserved in sterilized ice filled box and transported to the Bacteriology Lab of CASVAB.

ISO 16654 (2001) method was followed for detection of *E. coli* O157:H7. In brief 25 g of each sample was diluted in 225mL of modified tryptone soya broth (mTSB,

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Oxoid, UK), supplemented with novobiocin. The samples were then homogenized and incubated for 18–24 h at 41.5°C. After the enrichment the selective and differential isolation of enterohemorrhagic *E. coli* O157:H7 was carried out on Sorbitol MacConkey agar, supplemented with cefixime, and tellurite (CT-SMAC, Oxoid, UK) and incubated overnight at 37°C. From each sample, one Non Sorbitol fermenting (NSF) colony was transferred to tryptone soys agar (Oxoid) and incubated for 24 h at 37°C. Subsequently, one isolate from the subculture was further tested for agglutination with an E. coli O157:H7 latex agglutination test kit (Welcolex-Remel, UK) for serogroup O157:H7 confirmation.

ISO 6579 (2002) method was followed for detection of *Salmonella* spp. In brief each 25g food sample was diluted with 225mL of sterile Buffered Peptone Water (Oxoid - UK), homogenized and incubated for 18 h at 37°C. One milliliter of the culture was added to 10mL of Rappaport–Vassiliadis soy peptone and incubated at 42.5°C for 18 h. One loopful of the culture was then streaked onto xylose lysine deoxycholate agar (Oxoid, UK) and incubated at 37°C for 24h. The resulting presumptive *Salmonella* colonies were tested with biochemical screening on RapidID ONE test kits (Remel, UK)

For detection of *L. monocytogenes,* 25g of the food samples was mixed with 225mL of sterile Fraser Broth Listeria enrichment broth (Oxoid, UK), homogenized, followed by incubation for 48h at 30°C. One loopful of the culture broth was streaked onto Palcam agar (Oxoid, Hampshire, UK) and incubated at 37°C for 48 h. Presumptive colonies were streaked onto horse blood agar and TSA plates and incubated at 35°C for 48 h. The resulting presumptive *Listeria* colonies were submitted for biochemical screening (oxidase test, catalase test and Gram staining).

ISO 10272-1 (2006) method was followed for detection of *Campylobacter.* A 25 g of the food sample inoculated in 250mL of the enrichment medium Bolton Broth supplemented with 0.02g/L cefoperazone, 0.02g/L vancomycin, 0.02g/L trimethoprim lactate and 0.01g/L amphotericin-B without the addition of lysed defi brinated horse blood, which was incubated in a microaerobic atmosphere (5% O2, 10% CO2 and 85% N2) at 37°C for 4h to 6h, then at 41.5°C for 44 h (+/- 4h).

The enriched culture was inoculated with a sterile loop onto duplicate Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) supplemented with 0.032g/L cefoperazone and 0.01g/L amphotericin-B. Suspected colonies were taken from CCDA and streaked onto Blood Agar plates for confirmation. The plates were incubated in a microaerobic atmosphere at 41.5°C for 24h to 48h for examination of morphology, Gram staining, tests for catalase and oxidase, hippurate hydrolysis and hydrolysis identification of indoxyl acetate.

DNA was extracted through CTAB (Cetyletrimethyle ammonium bromide) method as described earlier by Minas *et al*. (2011).

PCR reactions mixture (30µl) consisted of 15µl of PCR Master mix (Gene All), 1µl of each primer (Macrogen), 10µl of Molecular grade water and 3µl of each extraction was used. Primers are enlisted in Table I. Amplification was performed in a gradient Thermocycler. Thermal cycler conditions were set as described by Shanmugasamy *et al*. (2011). An initial incubation at 94°C for 60 seconds, followed by 35 cycles of denaturation at 94°C for 60 seconds, annealing at 64°C for 30 seconds, elongation at 72°C for 30 seconds, and final extension period for 10 min at 72°C. A 50 bp DNA ladder (Gene One) was used. Deionized distilled water was used as a template for negative control.

Amplified PCR products were then electrophoresed in 1.2% Agarose w/v gel stained with Ethidium bromide and was documented in gel documentation apparatus (Salehi *et al*., 2005).

Table I.- List of oligonucleotide primers used.

Target gene	Primers (5' – 3')	Amplicon size	Reference
E.coli rfbO157	F: CGG ACA TCC ATG TGA TAT GG R: TTG CCT ATG TAC AGC TAA TCC	259 bp	Desmarchier *et al*. (1998)
E. coli fliCH7	F: GCG CTG TCG AGT TCT ATC GAG R: CAA CGG TGA CTT TAT CGC CAT TCC	625 bp	Pilpot and Ebel (2003)
C. jejuni mapA	F: CTA TTT TAT TTT TGA GTG CTT GTG R: GCT TTA TTT GCC ATT TGT TTT ATT A	589 bp	Denis *et al*. (1999)
L. mono hly	F: CAT TAG TGG AAA GAT GGA ATG R: GTA TCC TCC AGA GTG ATC GA	730 bp	Blais and Phillippe (1995)
Sal spp. invA	F: GTG AAA TTA TCG CCA CGT TCG GGC AA R: TCA TCG CAC CGT CAA AGG AAC C	284 bp	Oliveira (2003)

Results and discussion

Table II shows the prevalence of *Salmonella* spp., *Listeria monocytogenes, Campylobacter jejuni* and *E. coli* (O157:H7) in chicken meat, beef, milk and vegetables samples collected from market. Results revealed that out 0f 800 collected samples 48.37% were contaminated with above mentioned opportunistic bacteria. These results indicate alarming and poor food hygienic conditions prevalent in local market. *Campylobacter* was highest (28.99%) among the four investigated pathogens followed by *Salmonella* (19%), *E. coli O157:H7* spp. (8%) and *Listeria monocytogenes* spp. (1.25%), respectively. It was also found that fresh chicken meat compared to processed chicken, minced beef compared to diced beef, milk purchased from retailer compared to one from dairy farms, and fresh salad compared to fresh vegetables had higher bacterial load for all types of bacteria tested.

Table II.- Prevalence of foodborne pathogens in food items sold in local market.

Number of samples	*Salmonella* spp.	*C. j.*	*L. m.*	*E. coli* O157:H7
Chicken (n=100 each)				
Processed	24%	32%	1%	4%
Fresh	28%	48%	1%	8%
Beef (n=100 each)				
Diced	16%	20%	0%	8%
Minced/ground	20%	32%	2%	12%
Milk (n=100 each)				
From dairy farms	8%	16%	1%	4%
From retail markets	20%	28%	3%	12%
Vegetable (n=100 each)				
Fresh vegetables	4%	20%	0%	4%
Fresh salad	32%	36%	2%	12%
Total (n=800)	19%	28.99%	1.25%	8%

C. j., Campylobacter jejuni; L. m., Listeria monocytogenes.

The identification of bacteria was also confirmed by PCR product of 284 bp for *Salmonella,* 589 bp for *Campylobacter jejuni,* 730 bp for *Listeria monocytogenes* and 625 bp for *E. coli* (O157:H7) fragment of rbfO157 and 259 bp for flicH7 gene (Supplementary Fig. S1).

Salmonellosis is considered as the major foodborne illness globally and especially a chief cause of death in non developed countries. In this study, the overall prevalence of *Salmonella* in food items was found to be 19% ranging from 4-24% in different food items. These results are in line with the previous reports of 32%-36% prevalence of *Salmonella* in processed frozen chicken in Egypt and Nigeria (Adeyanju and Shola, 2014). Lower prevalence of *Salmonella* has been reported in developed countries like New Zealand (1.3%) and Italy (9.9%) (Wong *et al*., 2007; Saraj-Uddin *et al*., 2015).

Raw cooked food items can be contaminated with *Listeria*. Under normal circumstances lesterial infections causes diarrhea and gastroinstinal problems, leading to death in 20% cases. In this study, contamination with *Listeria* was significantly low to other investigated pathogens. Similar findings have been reported previously (de Silva *et al*., 2014). However, several studies have reported higher prevalence rate in samples of cheese sprouts and other fresh food items.

Campylobacteriosis is cited as the most common bacterial infections of humans globally. It leads body cramps, fever and pain accompanied with bloody diarrhea or dysentery. In this study 28.99% of food samples were found contaminated with *Campylobacter*, which put it on top of the list of pathogens investigated. Surprisingly, 32% processed and 48% fresh chicken meat samples were found positive for *Campylobacter*. Previously several studies have reported very high (91%) prevalence in fresh poultry meat (Wong *et al*., 2007).

Escherichia coli are mostly harmless and play an important role in healthy human digestive system, but some are equipped with virulence and pathogenic factors. To date six different *E. coli* pathotypes have been reported and Shiga toxin-producing *E. coli* O157:H7 is the most prevalent strain. Our results revealed the 8% prevalence of *E. coli* O157:H7 in 800 analyzed samples. Beef, milk and salad samples showed the highest rate (12%) of this infectious agent which are in agreement to previous studies (Chang *et al*., 2013; El-Tawab *et al*., 2015; Rahimi *et al*., 2012; Sancak *et al*., 2015). On contrary, high prevalence (48%) of *E. coli* was reported in raw vegetables in Multan, Pakistan (Rabia *et al*., 2014). In Lahore, another city of Pakistan 41-50% incidence of *E. coli* O157:H7 were found in juices, fresh fruits and vegetables (Mehwish and Ayesha, 2014). Chang *et al*. (2013) reported 3.3% of the prevalence of *E. coli* O157:H7 were found n in Malaysia which is in range of our findings.

Conclusion

This study indicates high prevalence of foodborne infectious in local market of Quetta, Pakistan. Special measures are required to improve the hygiene and quality of food items.

Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.4.sc17

Statement of conflict of interest

Authors have declared no conflict of interest.

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