Incidence, Enumeration and Confirmation of *Listeria* and its Species in Ready-to-eat Street Vended Salads Sold at Various Outlets of Faisalabad City, Pakistan

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**Abstract**

The desire for a healthy lifestyle and faster mode of preparation has supported the consumption of ready to eat fresh salad. Street vended salads are recognized as a source of pathogenic transamination in different parts of the world. The present study was designed to evaluate the safety status of fresh vegetable and Russian salads being sold at various food outlets of Faisalabad. Samples of freshly prepared salads were collected from representative selected different areas of Faisalabad city divided into four different zones (zone 1, zone 2, zone 3 and zone 4). Prevalence and enumeration of *Listeria* was done through microbial testing via the spread plate method. Among samples of vegetable salad, the highest prevalence of *Listeria* was found in the zone 2 (75%) whereas Russian salad samples from zones 1 and 3 exhibited 62% prevalence, the highest among all 4 zones of study. On the whole, the lowest prevalence of *Listeria* was found in zone 4 (50% vegetable salad and 58% Russian salad). Biochemical conformation of Listeria done through different tests for the identification of various *Listeria* species, exhibited that *Listeria monocytogenes* and *Listeria innocua* were highly prevalent in samples from zones 1 and 3 respectively. The results will help to improve safety concerns associated with street vended foods.

**Keywords:** *Listeria*, ready-to-eat, vended salads

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INTRODUCTION

Pathogenic microorganisms significantly exist in street vended foods and cause foodborne illnesses in all parts of the world. Foodborne illnesses not only affect consumer health but also result in negative economic impacts, loss of customers, product recall, and potential compensation costs. Contaminated foodstuff is the main cause of twenty to thirty percent spreading of Listeria infection that causes ninety-five percent cases of food poisoning. Every year, almost one third of the population is distressed with foodborne infections in industrialized countries whereas the problem is worse in developing countries due to poverty, overcrowding, inadequate sanitary conditions, and poor general hygiene.

Listeria is a Gram-positive, bacillus, non-spore forming, ubiquitous, motile, facultative anaerobe and one of the most environmental pathogenic microflora of food. Listeria can easily deteriorate the raw ready-to-eat foods because of its high salt tolerance and ability to thrive at low preservation temperatures. Listeria increases the rate of death up to 25-30% just because of hygienically poor environment and inadequate management of food processing. Due to consumption of contaminated and unhygienic foods nearly 76 million infections, 325,000 hospitalizations, and 5000 deaths are caused annually in United States of America.

Rapid change in the socioeconomic status and changing lifestyle have greatly increased the consumption of street vended foods. Control of safety of ready to eat street vended foods is difficult and much important because they have significant socioeconomic role in meeting food and nutritional requirements of consumers at affordable prices particularly to the lower and middle income people. As these food products are prepared by small street vendors, cheaper rates of these foods attract the low earners. Ready to eat street vended foods constitute the primary source of meals for many low and middle income people.

Salads are economical, convenient in preparation and have delicious taste but the hygiene and safety levels are big challenges for the food administrators. Freshly consumed produce got contaminated on farm or point of sale in market. Reported sources of contamination include soil, handling during pre and post-harvest handling, water and dust. Manures used to promote the growth and cultivation of these produce harbor the large number of pathogenic microorganisms such as Salmonella, Mycobacterium, Listeria monocytogenes, Klebsiella and Clostridium perfringens. Globally, about 325,000 people are hospitalized along with death of 5,000 children each year due to foodborne illnesses. Therefore, despite the freshness and nutritional benefits of fruits and vegetables, there have been an increase in incidence of foodborne illnesses thus bacteriologically safe fruits and vegetable products are required to maximize the health benefits promised by their consumption. Ingredients of salads are contaminated with soil, dust, irrigation water which are proved to be associated with pathogenicity of Listeria. Findings have reported the highest prevalence of Listeria monocytogenes in ready to eat salad samples followed by chicken products, beverages, egg based products, beef, and seafood products. In salads, Listeria has been reported to be isolated from fresh produce such as parsley, cucumber, cabbage, sprouts and water cress and resulted in morbidity and mortality in various parts of the world. The average shelf life of ready to eats ranges from 5-7 days and after opening of package product can be stored for two days at refrigeration conditions. Highly perishable, favorable pH (6.0-7.0), poor abidance to decontamination procedures and temperature abuse can increase the risk associated with these products. In Pakistan street vendors to large restaurants are responsible for sale of ready to eat salads. Most common salads on retail sale include vegetable salad and Russian salad. Common vegetables used in vegetable salads include onion, cucumber, tomatoes, lettuce green pepper, radish, salt and spices commonly stored at ambient temperature during retail conditions. While ingredients in Russian salads include fresh or boiled vegetables, mayonnaise, cream and...
spices stored at refrigeration conditions. This study provides the prevalence data of *Listeria monocytogenes* for Vegetable salad and Russian salad. The objectives of this study were to assess microbiological quality of freshly prepared street vended salads and to get information regarding the major sources of contamination.

**MATERIALS AND METHODS**
Sample of Vegetable and Russian salads were collected from four different zones of the Faisalabad city (Table 1). A total of 192 samples were picked during five weeks. Samples were kept in strict hygienic conditions in sterile zipper polythene bags, stored in an icebox under controlled temperature and brought to the Food Microbiology and Microbiology Laboratory, National Institute of Food Science and Technology, University of Agriculture, Faisalabad within an hour of its collection. *Listeria* selective agar was used for microbial analysis. Samples were homogenized in a peristaltic blender (Seward Europe) at 200 rpm for 2 minutes. For this purpose, 25g salad along with 25 mL of peptone water was taken in specialized stomacher bags. Six serial dilutions were made by transferring 1 mL liquid sample from stomacher bag to the test tubes serial wise and transferred to the prepared Petri plates containing solidified *Listeria* Selective Agar Base (Oxoid). Spreading of the poured volume (0.1mL) was done using a sterile spreader and plates were allowed to dry for 15 minutes (Fig. 1). After drying, incubation was done for 24 hours at 37°C. After incubation, dark brown or blackish colored colonies grew over *Listeria* selective agar as depicted in Fig. 2. Colonies of *Listeria* were counted using the colony counter (galaxy 230-USA) and documented. Colony forming units per gram (CFU/g) were calculated using the following formula.

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**Table 1.** Sampling plan for vegetable and Russian salads collection

| Zone 1 locations for collection of samples | Zone 2 locations for collection of samples |
|-------------------------------------------|------------------------------------------|
| No. | Vegetable salad | Russian Salad | No. | Vegetable salad | Russian Salad |
|-----|-----------------|---------------|-----|-----------------|---------------|
| 1   | Yong Wala       | Ghulam Muhammadabad road | 1   | Canal road      | Sargodha road |
| 2   | Kotwali road    | Tata bazar    | 2   | Abdullahpur overpass | Millat road |
| 3   | Narwala road    | Sadar bazar   | 3   | Tahir road      | Fsd.-Sanghla hill road |
| 4   | Jail road       | Tariq road    | 4   | Khaleeq road    | Kashmir road  |
| 5   | Ganda nala road | Gulberg road  | 5   | Noorpur road    | Iqbal stadium |
| 6   | Bakar mandi road | Makki road   | 6   | Chibban road    | Lhr.-Fsd. Shk. road |
| 7   | Chiniot bazar   | Kachari bazar | 7   | Shadman road    | College road  |
| 8   | Oil depot road  | Imam bargha road | 8   | Ittehad road    | Wapda colony road |

| Zone 3 locations for collection of samples | Zone 4 locations for collection of samples |
|-------------------------------------------|------------------------------------------|
| No. | Vegetable salad | Russian Salad | No. | Vegetable salad | Russian Salad |
|-----|-----------------|---------------|-----|-----------------|---------------|
| 1   | Rasool park     | Susan road    | 1   | Liaqat abad     | Samundari road |
| 2   | Tufail shaheed road | Tezab mill road | 2   | Mulyanwala road | D-type colony road |
| 3   | Younas road     | Jaranwala road | 3   | Naimatabad road | Dijkot road |
| 4   | Satyana road    | D-ground      | 4   | Sanni road      | Samanabad road |
| 5   | Maqbool road    | Lower canal road | 5   | Main road       | Railway road |
| 6   | Usman road      | Khawaja islam road | 6   | Saifabad road   | Jhang road |
| 7   | Allama Iqbal road | Illahi abad road | 7   | Talibanwala road | AARI colony road |
| 8   | Risalewala road | Warispura road | 8   | Bilal road      | Fazal elahi road |

Samples were collected in triplicate from each location.
CFU/g = (Average No.of Colonies x Dilution Number ) / (Dilution Factor x Volume plated )

(Formula A)

DF = (Volume of sample) / (Sample Volume + Diluent Volume)

(Formula B)

Biochemical Testing
Two species of *Listeria* (*Listeria monocytogenes* and *Listeria innocua*) were identified and biochemically confirmed by through Gram staining, catalase, citrate, hydrogen sulfide, indole, methyl red and oxidase tests as described by the Laboratory Manual of Food Microbiology. Statistical analysis was performed for to determine the significance of test results by using the completely randomized design with the help of Statistix 8.1 software.

RESULTS AND DISCUSSION

Vegetable Salad

Prevalence of *Listeria* in street vended vegetable salad samples gathered from zone 1, zone 2, zone 3 and zone 4 are shown in Table 2. Total of 24 samples of vegetable salad were collected separately from all four zones and the occurrence of *Listeria* was documented in percentage. Among 24 samples of vegetable salad 15 samples of vegetable salad illustrated positive results for the pathogen and the total prevalence of *Listeria* was recorded 62%. The highest count of 1.59x10^8 CFU/g was recorded from Ganda Nala Road and the lowest count of 4.47x10^6 CFU/g was recorded from oil depot road (Table 3). Highest prevalence was found in the area of Yong wala and Narwala road whereas *Listeria* was not detected from the samples of Chiniot bazar. The following results are in accordance with the findings of

| Locations | Total prevalence in vegetable salad | Total prevalence in Russian salad |
|-----------|-------------------------------------|----------------------------------|
| Zone 1    | 15/24 (62%)                         | 15/24 (62%)                      |
| Zone 2    | 18/24 (75%)                         | 11/24 (45%)                      |
| Zone 3    | 13/24 (55%)                         | 15/24 (62%)                      |
| Zone 4    | 12/24 (50%)                         | 14/24 (58%)                      |

*Table 2. Prevalence of Listeria in salad samples from different zones*

![Fig. 1. Plates poured with *isteria selective agar*](image1)

![Fig. 2. Brown colored colonies of *Listeria* grown on Plates.](image2)
Table 3. Prevalence and enumeration of *Listeria* in vegetable salad

| Zones | Location               | Satisfactory | Un satisfactory | Colony forming unit (CFU/g) |
|-------|------------------------|--------------|-----------------|-----------------------------|
|       |                        | Samples % (<10^7 CFU/g) | Samples % (>10^7 CFU/g) |                              |
| 1     | Yong Wala              | -            | 100             | 1.30x10^7                   |
|       | Kotwali road           | 33           | 66              | 6.19x10^6                   |
|       | Narwala road           | -            | 100             | 8.42x10^6                   |
|       | Jail road              | 33           | 66              | 1.12x10^6                   |
|       | Ganda nala road        | 33           | 66              | 1.59x10^6                   |
|       | Bakar mandi road       | 33           | 66              | 9.4x10^5                    |
|       | Chiniot bazar          | 100          | -               | ND*                         |
|       | Oil depot road         | 66           | 33              | 4.47x10^6                   |
| 2     | Canal road             | 66           | 33              | ND*                         |
|       | Abdullah pur overpass  | 33           | 66              | 5.61x10^6                   |
|       | Tahir road             | -            | 100             | 1.80x10^7                   |
|       | Khaleeq road           | 33           | 66              | 7.06x10^7                   |
|       | Noorpur road           | -            | 100             | 3.86x10^6                   |
|       | Chibban road           | 33           | 66              | 8.73x10^7                   |
|       | Shadman road           | -            | 100             | 1.40x10^6                   |
|       | Ittehad road           | 33           | 66              | 6.20x10^6                   |
| 3     | Rasool park            | 33           | 66              | 4.60x10^6                   |
|       | Tufail shaheed road    | 66           | 33              | 2.80x10^7                   |
|       | Younas road            | 33           | 66              | 6.39x10^6                   |
|       | Satyana road           | 66           | 33              | 4.86x10^6                   |
|       | Maqbool road           | -            | 100             | 2.47x10^7                   |
|       | Usman road             | 33           | 66              | 1.06x10^6                   |
|       | Allama iqbal road      | 33           | 66              | 3.56x10^6                   |
|       | Risalewala road        | 33           | -               | ND*                         |
| 4     | Liaqat abad            | 66           | 33              | 6.73x10^6                   |
|       | Mulyanwala road        | 33           | 66              | 8.46x10^5                   |
|       | Naimatabad road        | 33           | 66              | 3.73x10^5                   |
|       | Sanni road             | 66           | 33              | 4.93x10^5                   |
|       | Main road              | 100          | -               | ND*                         |
|       | Saifabad road          | -            | 100             | 2.99x10^7                   |
|       | Talianwala road        | 33           | 66              | 6.28x10^6                   |
|       | Bilal road             | 66           | 33              | 1x10^7                      |

Christson et al\(^a\) who reported the presence of *Listeria monocytogenes* in street vended salad which may cause health hazards.

In zone 2, prevalence of the pathogen was recorded as 75\% in vegetable salad. Total 18 samples were positive for *Listeria* as shown in Table 3. Tahir road, Noorpur road and Shadman road have shown highest prevalence while no pathogen was detected in samples taken from Canal road with the non-significant difference (P>0.05). Enumeration results indicated highest count of 1.40x10^8 in samples from Shadman road while the lowest count of 3.86x10^6 CFU/g was recorded in samples collected from Noorpur road. The following results are in agreement with the study reported by El-Shenawy et al\(^a\) according to which *Listeria* is present everywhere in our environment and its various species like *Listeria monocytogenes* is responsible for many outbursts. In zone 3, overall 55\% prevalence was observed in vegetable salads. None of the sample was found acceptable for the consumption. Highest count of 1.06x10^8 CFU/g was found in the area of Usman road whereas lowest count was shown in the area of Satyana road (4.86x10^6 CFU/g) with no detection in the area of Risalewala road as presented in the Table 3. The following results are in accordance with the study of Lianou, & Sofos\(^a\) who stated that inefficient methods of handling of street vended salads can support the growth of pathogenic microorganisms.
flora in street vended foods. Most of the vendors openly store leftover food in an open place for the next day use. Some of them also use stagnant water for washing which in result can cause serious health hazards.

In zone 4, vegetable salads have shown the total prevalence of 50%. Highest count was found in the area of Saifabad road that is 2.99x10^7 CFU/g whereas lowest count was observed in the samples taken from Naimatabad road (3.73x10^5 CFU/g). No pathogen was detected in the Main road samples. The results are in agreement with the work of Gelfand and colleague who describes the sources for the high prevalence of Listeria which may include the dusty unygenic premises and poor sanitary conditions.

**Russian Salad**

Result of the study (Table 2) presented the significant difference in samples of Russian salad in zone 1 and zone 3 with 62% unsatisfactory results. None of the samples was found to be in the acceptable limits. In zone 1, highest count of 1.30x10^8 CFU/g from Makki roadand the least count of 3.86x10^5 CFU/g were observed in the area of Ghulam Muhammadabad road whereas no Listeria was detected in the samples taken from

| Zones | Location                          | Satisfactory Samples % (<101 CFU/g) | Un satisfactory Samples % (>102 CFU/g) | Colony forming unit (CFU/g) |
|-------|----------------------------------|-------------------------------------|----------------------------------------|----------------------------|
| 1     | G.M Abad road                    | 66                                  | 33                                     | 3.86x10^5                  |
|       | Tata bazar                       | 100                                 | -                                      | ND*                        |
|       | Sadar bazar                      | 33                                  | 66                                     | 1.26x10^7                  |
|       | Tariq road                        | 33                                  | 66                                     | 1.16x10^8                  |
|       | Gulberg road                     | -                                   | 100                                    | 2.62x10^6                  |
|       | Makki road                        | 33                                  | 66                                     | 1.30x10^8                  |
|       | Kachari bazar                    | 33                                  | 66                                     | 3.73x10^6                  |
|       | Imam bargha road                 | 66                                  | 33                                     | 4.13x10^6                  |
| 2     | Sargodha road                    | 100                                 | -                                      | ND*                        |
|       | Millat road                      | -                                   | 100                                    | 1.84x10^7                  |
|       | Fsd.-Sanghla hill road           | 33                                  | 66                                     | 7.66x10^5                  |
|       | Kashmir road                     | 66                                  | 33                                     | 7.40x10^6                  |
|       | Iqbal stadium                    | 100                                 | -                                      | ND*                        |
|       | Lhr Fsd Shk road                 | 66                                  | 33                                     | 5.13x10^7                  |
|       | College road                     | -                                   | 100                                    | 2.99x10^8                  |
|       | Wapda colony road                | 66                                  | 33                                     | 4.33x10^6                  |
| 3     | Susan road                       | 100                                 | -                                      | ND*                        |
|       | Tezab mill road                  | -                                   | 100                                    | 2.63x10^6                  |
|       | Jaranwala road                   | 33                                  | 66                                     | 8.87x10^6                  |
|       | D-ground                         | 33                                  | 66                                     | 2.33x10^8                  |
|       | Lower canal road                 | 33                                  | 66                                     | 4.44x10^7                  |
|       | Khawaja islam road               | 33                                  | 66                                     | 2x10^8                     |
|       | Illahi abad road                 | 33                                  | 66                                     | 5.75x10^7                  |
|       | Waris pura road                  | 33                                  | 66                                     | 9.40x10^7                  |
| 4     | Samundari road                   | -                                   | 100                                    | 1.67x10^7                  |
|       | D-type colony road               | 100                                 | -                                      | ND*                        |
|       | Dijkot road                      | 33                                  | 66                                     | 7.14x10^6                  |
|       | Samanabad road                   | 33                                  | 66                                     | 7.01x10^6                  |
|       | Railway road                     | 33                                  | 66                                     | 1.21x10^7                  |
|       | Jhang road                       | 33                                  | 66                                     | 4.33x10^6                  |
|       | AARI colony road                 | 66                                  | 33                                     | 3.33x10^5                  |
|       | Fazal elahi road                 | 33                                  | 66                                     | 1.06x10^4                  |
Tata bazar with the overall count ranging from 105-10^8 CFU/g. These results are closely related to the previous work done by Razzaq and colleagues, which reports that fresh and mixed salads sold by road merchants harbor numerous pathogenic micro flora.

In zone 2, the percentage of total prevalence in Russian salad samples was 45% as documented in the Table 2. Enumeration of Listeria in Russian salad samples collected from zone 2 shown the significant results and the highest count was found in the area of College road with 2.99x10^6 CFU/g while the lowest count of Listeria was recorded in samples taken from Faisalabad-Sanghla hill road (Fsd.-Sanghla hill road) as 7.66x10^5 CFU/g. No Listeria was detected in the samples gathered from the areas of Sargodha road and Iqbal Stadium as described in Table 4. These results are in accordance with the results of research in which shows that by adopting good hygiene practices like use of hygienic tops, aprons, utensils and good sanitary conditions can reduce the risk of Listeria by half.

Similar to zone 1, percentage of total prevalence of Listeria in Russian salad samples was 62% with the highest count in the area of Fazal Elahi road with 1.06x10^6 CFU/g count while the least count was recorded in the samples taken from AARI colony road (3.3x10^5 CFU/g). No Listeria was detected in the area of D-type colony road (Table 4). The results are in agreement with the work of Gelfand and coworkers that describes the sources for the high prevalence of Listeria. Similarly in another study conducted in Malaysia commercially available minimally processed vegetable salads were analyzed microbiologically and Listeria monocytogenes and Listeria spp. were detected in 22.5% and 33.3% samples respectively.

**Biochemical Testing**

Biochemical conformation for the identification of various Listeria species (resulted shown in Table 5) indicated that the percentage of Listeria monocytogenes in vegetable salad and Russian salad was 62% and 45% respectively in the samples collected from zone 1, while the occurrence of Listeria innocua in the samples of vegetable salad and Russian salad was 16% and 8%. In the zone 2, the prevalence of Listeria monocytogenes in vegetable and Russian salad samples was 58% and 41% while that of Listeria innocua was 16% and 8% in vegetable and Russian salad samples respectively. In zone 3, the total percentage of Listeria monocytogenes in vegetable and Russian salad was 37% and 45%, whereas the percentage of Listeria innocua was 16% and 12% respectively. Similarly, in the zone 4, the occurrence of Listeria monocytogenes 41% and 45% while in case of Listeria innocua the prevalence was recorded as 16% and 8% in vegetable and Russian salad samples respectively. A study conducted in Brazil on 162 minimally processed vegetable salad samples sold locally also showed similar results containing Listeria monocytogenes, Salmonella and Escherichia coli at levels above (World Health Organization) WHO recommended limits. In present study high Listeria count indicates increased consumer’s exposure to microorganisms as it is assumed that the pathogen level were not reduced from post salad production to the utilization point because salads were served as ready to eat form and were stored at room temperature for sale. Possibility is that rework salad from last day was also mixed in new lot to utilize it risking the overall quality of product. Therefore holding temperature...
and Thermal processing have strong impact on microbiological safety. The quality and safety of vegetables used for ready to eat salad preparation also depend upon use of clean irrigation water and appropriate washing to remove residues of soil and organic fertilizers. Thus for the prevention of increased bacterial load application of good hygiene practices, effective control on storage condition, decontamination and selection of appropriate packaging material is necessary.

CONCLUSION

The study revealed that the microbiological quality of salads sold at various markets of Faisalabad were unwholesome for human consumption. On overall basis, *Listeria monocytogenes* was the most prevalent specie of *Listeria* that was found in most of the samples of salads collected from all zones. On the other hand, the prevalence of *Listeria innocua* was very low as compared to *Listeria monocytogenes*.

The contamination could be attributed to the production source of vegetables, use of contaminated water for washing, improper handling, use of dirty processing utensils, knives and trays, poor personal hygiene and improper storage conditions generally along environmental conditions and cross contamination from rotten vegetables in value chain. Simple washing of vegetables before use can possibly reduce the risk of microbiological hazards. The need is to provide basic food hygiene training to food handlers and to create consumer awareness to consume properly processed food products. Further it is recommended that research should be carried out on salads to isolate bacterial pathogenic strains identified in present study and antimicrobial susceptibility of identified bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All data set generated or analyzed during the study are included in the manuscript.

ETHICS STATEMENT

This research does not contain any studies with human participants or animals performed by any of the authors.

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