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Microbiological Analysis of Street Vended Vegetables in Multan City, Pakistan: A Public Health Concern

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Abstract.- This study was aimed to assess the microbiological quality of mixed and separate fresh vegetables and to observe the potential hazards to public health. One hundred and forty five samples of street vended raw vegetables, sold outside authorized market areas for immediate consumption, were screened for bacterial identification and antibiotic sensitivity pattern. Fresh vegetables of street vendors were harboring many microbial contaminants and pathogens. The average total aerobic plate counts cfu per gram (n = 3) in raw mixed vegetables, radish, cabbage, carrot, cucumber, coriander and tomato samples were determined as 2.1×10^{9}, 2.0×10^{7}, 3.0×10^{7}, 4.0×10^{4}, 4.0×10^{4}, 6.9×10^{8}, 4.0×10^{7}, and the average total coliform plate count cfu per gram (n = 3) was 3.2×10^{7}, 5.2×10^{3}, 9.0×10^{2}, 3.0×10^{4}, 5.0×10^{3}, 3.5×10^{5}, and 3.0×10^{8}, respectively. The prevalence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Enterobacter* spp. and *Salmonella* spp. was found to be 48%, 60%, 48%, 64% and 8%, respectively in the raw vegetables (mixed and separate). All the strains were sensitive to ampicillin, erythromycin, kanamycin and nalidixic acid. The high microbial contamination rates associated with these vegetable samples indicated that overall agricultural, hygiene, harvesting, production and sale practices are poor in the studied area. The consumers should be alarmed of this situation, and thus should adopt suitable sanitary approaches in this context to avoid health problems.

Key words: *Enterobacter* spp., microbial load, *Salmonella* spp., antibiotic susceptibility pattern, *S. aureus*, *P. aeruginosa*

INTRODUCTION

In Pakistan, there is an increasing consumption of raw fresh vegetables, fruits and sprouts. Vending of cut salad vegetables, fruits and sprouts is common practice in Pakistan. Outbreaks of human diseases due to the transmission of pathogenic microorganisms on vegetables usually result from fecal contamination which is due to the use of raw sewage or manure fertilizers, contaminated water for irrigation, and washing with contaminated ice for transport and unhygienic handling (Brackett and Splittstoesser, 1992). Street foods provide a source of readily available, inexpensive, nutritional meals, and are associated with the outbreaks of food-borne diseases in many countries of the world (Wachtel *et al*., 2002). Outbreaks of human gastroenteritis have been associated with the consumption of contaminated fresh vegetables (De Roever, 1998).

Staphylococcus aureus is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when observed under microscope and has large, round, golden-yellow colonies. *S. aureus* can cause range of illnesses from minor skin infections, such as pimples, boils, impetigo, folliculitis, cellulitis, carbuncles, furuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, osteomyelitis septicemia and toxic shock syndrome (TSS). Enterobacter is a genus of common Gram-negative, facultatively-anaerobic, rod-shaped bacteria of the family Enterobacteriaceae. The important members of this family are *Escherichia coli*, *Salmonella*, *Shigella* and *Klebsiella*. *Enterobacter* is a genus of common Gram-negative, facultatively-anaerobic, rod-shaped bacteria of the family Enterobacteriaceae. *Salmonella* spp. is an important cause of gastrointestinal illness in human. *S. enteritidis* and *S. typhimurium* are important species of *Salmonella* spp. The urinary and respiratory tract are the most

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common sites of infection. Salmonellosis is characterized by diarrhea, abdominal cramps, fever, and vomiting usually lasting 4-7 days (Collin *et al*., 1995). *E. coli* is Gram-negative, facultative anaerobic and non-sporulating bacterium and is always considered to be of faecal origin. It exists only transiently in other environment. Virulent strains of *E. coli* can cause gastroenteritis, neonatal meningitis and urinary tract infections. In some cases, virulent strains are also responsible for haemolytic-uremic syndrome, mastitis, septicemia, peritonitis and Gram-negative pneumonia. *P. aeruginosa* is a Gram-negative, aerobic, rod-shaped bacterium measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other infections (Naz *et al*., 2013).

This study was aimed (i) to assess the microbiological quality of mixed and separate fresh vegetables, (ii) to observe the potential hazard of street vended salad vegetables for public health and (iii) to determine the susceptibility patterns of the isolates obtained from fresh vegetables against antibiotics using disc diffusion method.

MATERIALS AND METHODS

Sample collection

The present study was carried out during 31.01.2010 to 30.01.2011. During this period, one hundred and forty five samples were collected from street vendors, in the form of raw vegetables (mixed and separate), such as carrot, coriander, cucumber, radish, cabbage, and tomato, sold outside authorized market areas in Zip-lock bags and transported to the laboratory on ice and analyzed within 1-2 h of procurement from different parts of Multan city.

Twenty five grams of each mixed vegetable sample were weighed aseptically in a sterile stomacher bag and homogenized with 225 ml of buffered peptone water for 2 min. The mixture was kept for 1 h at room temperature before analysis, unless otherwise stated for particular determinations. Serial dilutions (from 10^{-1} to 10^{-10}) of the homogenized samples were made before analysis.

Culturing and microbiological analysis

Aerobic plate counts were done by the pour plating method on plate count agar, followed by incubation at 30°C for 48 h; colonies were recorded as cfu/g (Viswanathan and Randhir, 2001; Mehmet and Aydin, 2008). Total coliforms were enumerated on violet red bile agar (Viswanathan and Randhir, 2001). The isolation and enumeration of *S. aureus* was done by pour plating the serial dilutions onto Baird Parker Agar containing egg yolk and then coagulase test was performed to test the presence of coagulase-positive staphylococci (Hasan *et al*., 2006). MacConkey agar was used for isolation and identification of lactose fermenting, Gram-negative enteric pathogens. Well-isolated colonies were initially Gram stained and then biochemically characterized. For Gram-negative bacteria, MICROBACT™ 24E (OXOID) was used for the identification of Gram-positive bacteria identified by Gram staining and, catalase, DNase and coagulase test by standard methods (Al-Mohizea, 1996).

Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 12.0 (SPSS, Chicago, IL, USA) software was used for the statistical analysis of the data. Two-way ANOVA and DMRT were applied on the data.

RESULTS AND DISCUSSION

Enumeration of organisms

The average total aerobic plate counts cfu per gram (n = 3) in raw mixed vegetables, radish, cabbage, carrot, cucumber, coriander and tomato samples were determined as 2.1×10^{9}, 2.0×10^{7}, 3.0×10^{7}, 4.0×10^{4}, 4.0×10^{4}, 6.9×10^{8}, 4.0×10^{7}, and the average total coliform plate count cfu per gram (n = 3) was 3.2×10^{7}, 5.2×10^{3}, 9.0×10^{2}, 3.0×10^{4}, 5.0×10^{3}, 3.5×10^{5}, and 3.0×10^{8}, respectively.

Prevalence of micro-organisms in raw vegetables (mixed and separate)

Vegetables and fruits get contaminated with pathogenic microorganisms and grow in fields or orchards or during harvesting, post harvesting

handling, processing and distribution. The potential hazard of street-vended salad vegetables have been reported previously (Houang *et al*., 1991). In the handling practices usually by vendors and the environment of displaying cut salads, there is possibility of food borne disease outbreaks. In present study, the samples tested showed aerobic plate counts in the range of 10^4-10^9 cfu/g and total coliform counts are in the range of 10^2-10^7 cfu/g. In a similar study, authors investigated aerobic plate counts in the range of 10^5-10^{12} cfu/g and total coliform counts in the range of 10^2-10^{11} cfu/g (Charles *et al*., 1976). Accordingly, these can be considered to be belonging to the spoiled grade and hence unsafe for consumption. Different studies revealed different aerobic and coliform plate counts (Pingulkur *et al*., 2000; Tambekar and Mundhada, 2006; Kapperud *et al*., 1995). In present study, prevalence of *S. aureus* was highest in samples of cucumber, followed by cabbage, radish, coriander, carrot and tomato. The isolation of *Staphylococcus* has been reported earlier (Levy, 1983; Olayemi, 1997).

In present study, *S. aureus*, *P. aeruginosa*, *E. coli*, *Enterobacter* spp. and *Salmonella* spp. in mixed vegetables were found to be 48%, 60%, 48%, 64% and 8%, respectively. Similar results have been observed in another study (Houang *et al*., 1991) involving *S. aureus*, *E. coli*, *Enterobacter* spp., *Klebsella* sp., *Providencia* spp. and *P. aeruginosa*. Another study reported similar results (Angulo *et al*., 2000) involving faecal coliforms, listeria and yersinia. Whereas, Tambekar and Mundhada found *E. coli* (38.3%), enetrobacter (20.9%), *Pseudomonas* spp. (16.2%), *S. aureus* (15.1%), *Salmonella* spp. (5.8%) and *Shigella* spp. (3.4%). We observed highest carriage rate of *P. aeruginosa* in tomato, followed by carrot, cucumber, cabbage, and coriander. Recovery of enteric bacteria and *P. aeruginosa* from vegetable salad has been reported earlier (Olayemi, 1997). In this study, it was revealed that there was significant ($p<0.05$) difference among different vegetable groups for the prevalence of various bacteria. Similarly, the prevalence of bacterial isolates were also significantly ($p<0.05$) variable among all vegetable types. DMRT revealed a significant difference between various bacteria for mixed vegetables. *P. aeruginosa* was significantly ($p<0.05$) highest for its prevalence in mixed vegetables, radish and cabbage. However for carrots, *Enterbacter* spp. had significantly ($p<0.05$) high prevalence.

In present study, there was a highest carriage rate of *E. coli* in radish (50%) and cabbage (50%), followed by carrots (25%) and coriander (25%). Samples from cucumber and tomato showed no growth for *E. coli*. The prevalence rate of *Salmonella* spp. was highest in radish (50%) followed by cabbage (25%) and carrots (25%). On the other hand, Kapperud *et al*. (1995) detected salmonellae (7.5%) in the samples of vegetables from farms, a wholesale market, supermarkets and small shops. There are reports of antibiotic resistance among food borne pathogens isolated from vegetables and fruits (Olayemi, 1997). The isolates of *E. coli*, *Enterobacter*, *Salmonella*, *S. aureus* and *P. aeruginosa* were found to be multi-drug resistant. This increases the potential risk of a growing number of antibiotic resistant organisms in developing countries like Pakistan, where polluted water is used for irrigation and sewage sludge as fertilizers. In our study, *S. aureus* obtained from fresh vegetables showed highest resistance to ceftriaxone (48%) followed by oxacillin (38%), gentamicin (29%), while all the strains were sensitive to ampicillin, erythromycin, kanamycin and nalidixic acid. In another study, it was found that *S. aureus* was sensitive to ampicillin, kanamycin, carbenicillin, ciprofloxacin and cefotaxime (Houang *et al*., 1991).

We observed that most of the isolates of Enterobacteriaceae were multidrug resistant. *Salmonella* spp. and *P. aeruginosa* were 100% resistant to ampicillin. Enterobacter showed the highest resistance to ampicillin (58%) (Fig. 1). All the strains of *E. coli*, *Salmonella* and *Enterobacter* were 100% sensitive to gentamicin and ceftriaxone, while erythromycin, kanamycin and nalidixic acid showed 64%, 48% and 69% sensitivity, respectively. *E. coli* was highly susceptible to various antibiotics showing 81% sensitivity to ampiciilin, 93% sensitivity to erythromycin, 100% sensitivity to gentamicin, 90% sensitivity to ceftriaxone, 81% sensitivity to kanamycin, and 93% sensitivity to nalidixic acid. *Enterobacter* showed

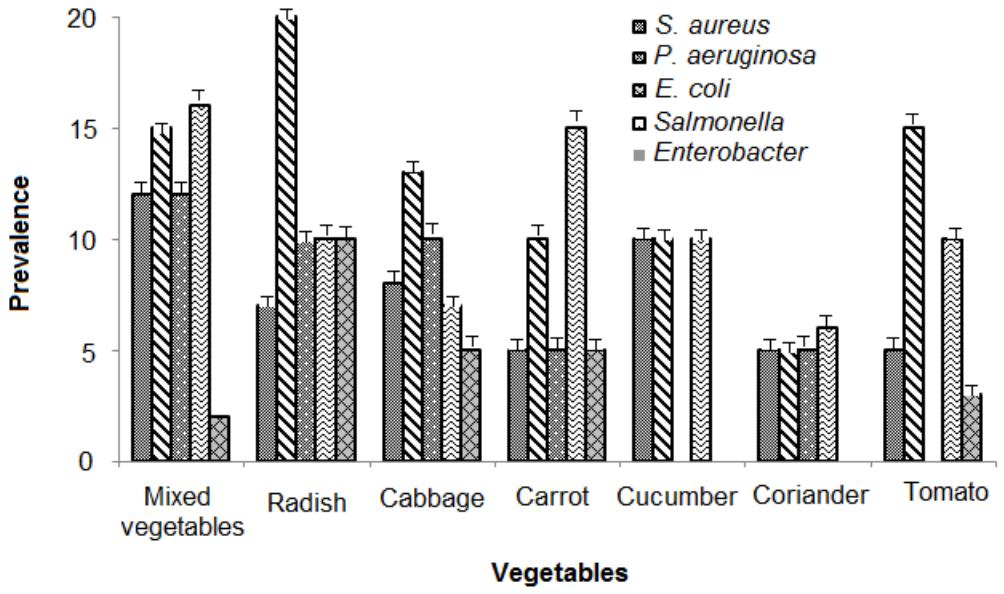

Fig. 1. Prevalence of various microorganisms in different vegetables

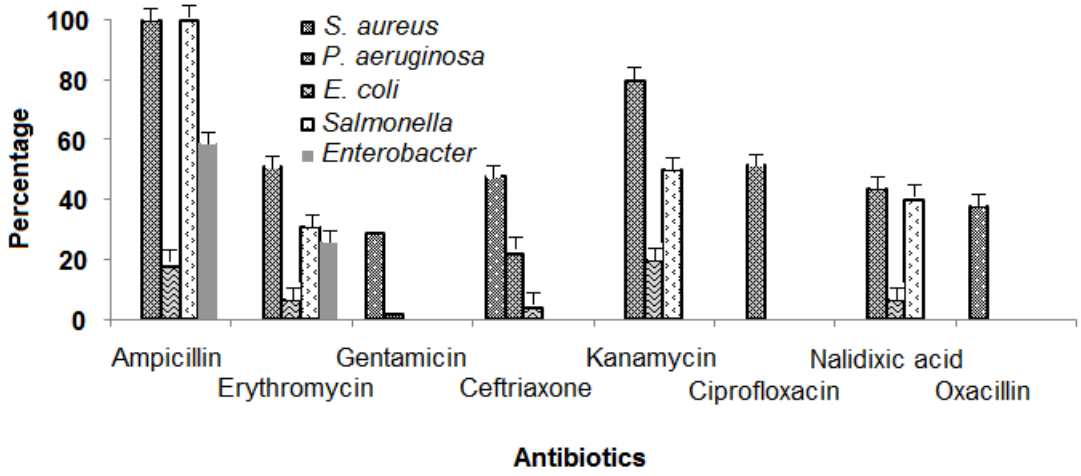

Fig. 2. Antimicrobial susceptibility data of isolated microorganisms against different antibiotics.

100% sensitivity to gentamicin, ceftriaxone, kanamycin and nalidixic acid, while 39% were sensitive to ampicillin and 67% were sensitive to erythromycin. *P. aeruginosa* showed 100% resistance to ampicillin, while 47%, 90%, 74%, 17% and 52% of the isolates were sensitive to erythromycin, genatmicin, ceftriaxone, kanamycin, and nalidixic acid, respectively. Viswanathan and Randhir reported that most of the isolates of Enterobacteriace and *P. aeruginosa* were resistant to ampicillin (Houang *et al*., 1991; Khan *et al*., 2013). *E. coli* was most resistant to carbenicillin (62.8%); *Enterobacter* to ampicillin (59.6%) and carbenicillin (65.4%); *Salmonella* to ampicillin (100%); *P. aeruginosa* to ampicillin and nalidixic acid (100%) and kanamycin (98.6%); and *S. aureus* to nalidixic acid (100%). The members of family *Enterobacteriacea*, *E. coli*, *Enterobacter*, *Serratia* and *Salmonella* were sensitive to amikacin. *E. coli* and *P. aeruginosa* showed resistance to

chloramphenicol. In present study, *Salmonella* spp., isolated from fresh vegetables, showed 100% resistance to ampicillin. All the strains were 100% sensitive to gentamicin and ceftriaxone, while erythromycin, kanamycin and nalidixic acid showed 64%, 48% and 69% sensitivity, respectively. Since most salmonella infections are acquired from ingestion of contaminated foods of animal origin, a likely cause for the increasing incidence of antimicrobial-resistant *Salmonella* spp. is the use of antimicrobial agents in food animals (Angulo *et al*., 2000; Mahmood *et al*., 2012; Rashid *et al*., 2012).

CONCLUSIONS

The data presented here show that the analyzed samples had microbial load which could be hazardous for public health. Fresh vegetables of street vendors are harboring many microbial contaminants and pathogens. The high microbial contamination rates associated with these vegetable samples indicate that overall sale practices are poor. The study revealed the potential hazard of street vended salad vegetables, considering the handling practice usually carried out by vendors and environment in which they display salad vegetables and the possibility of food related outbreaks of disease; vigorous washing of vegetables with safe running water before consuming required to reduce the number of microorganisms. The government intervention is also required to protect consumers and to ensure that the standard of safety of such foods is attainable in context of prevailing local situation. The consumers should be alarmed of this situation, and thus should adopt suitable sanitary approaches in this context to avoid health problems.

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