

## Full Length Research Paper

# Prevalence and antibiotics susceptibility patterns of some bacterial isolates from a street vended fruit product

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The study is aimed to evaluate the food safety status of a street vended fruit product locally known as fruit chat. Sample collection was carried out from four groups of vendors which were discriminated based on their mobility, vending practice and storage facility. The extent of prevalence for *Enterobacter* species, *Escherichia coli*, *Klebsiella* species, *Salmonella* species, *Staphylococcus aureus* and *S. epidermidis* was assessed in fruit chat samples. Bacterial isolates were identified through biochemical characterization. The degree of susceptibility of these isolates was evaluated against six different antibiotics that is, amikacin, ampicillin, ciprofloxacin, gentamycin, ceftriaxone and co-trimoxazol using disc diffusion method. Results indicated a higher ( $10^5$  to  $10^{10}$  CFU/g) total plate count (TPC) and total coliforms count (TCC) ranged from  $10^4$  to  $10^9$  CFU/g in samples collected from mobile vendors without covering. Mobility of vendors and, use of no covering, resulted in significant ( $P \leq 0.05$ ) contamination. However, fruit chat samples collected from stationary vendors with refrigeration facilities exhibited considerably lower bacterial count. The study clearly indicated that consumption of fruit chats from street vendors can be a potential risk for food borne outbreaks because of their contamination level by pathogenic bacteria. Bacterial species had shown variable susceptibility and resistance patterns in response to different antibiotics used in the study. However, all bacterial species had shown greater sensitivity for amikacin, whereas, bacterial strains appeared to develop resistance against other commonly used antibiotics. The mounting resistance against antibiotics is currently one of the foremost challenges to treat food borne infections. Therefore, both preventative and effective curative measures should be adopted. Firstly, the safety status of street vended food products can be enhanced by improving hygienic conditions during the preparation of food stuff. Secondly, the use of effective antibiotics will be of significant importance to cure food borne infections as well as to avoid growing resistance in pathogenic bacteria.

**Key words:** Safety status, street vended fruit product, bacterial prevalence, antibiotics susceptibility.

## INTRODUCTION

In developing countries like Pakistan, street vending of food items is very common because of their convenient availability and low cost. Thus, consumption of such food products has increased considerably and has become an important diet for a large number of populations. Hence, millions of people in the country consume a wide variety of street vended food items. Though, street vending

provides employment opportunities to local population especially in situations where initial investment is low. Moreover, men and women involved in street vending do not require particular skills and education (WHO, 2010). Food poisoning caused by various food items that are sold or served at public places have been well reported (Latham, 1997; Muleta and Ashenafi, 2001). Several such incidents from both developing and developed countries have been described. For example, in Senegal, more than 200 cases of food poisoning were recorded and street vended dairy products were incriminated (Dawson and Canet, 1991). Likewise, *Staphylococcus*

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*aureus* was found to be a causative agent for an outbreak of acute gastroenteritis in Australia as a result of contaminated food (Schmid et al., 2007). More recently, a major food poisoning outbreak in Pakistan occurred in a flood relief camp where one child died and over 300 people suffered after eating contaminated food (Anonymous, 2010).

Microbiological studies from many developing countries, carried out on street vended food articles have revealed a high bacterial count. *Salmonella* species, *S. aureus* and members of the family *Enterobacteriaceae* were common pathogens found in such food items (Bryan et al., 1997; Mosupye and von Holy, 1999).

A combination of different seasonal fruits and vegetables with added spices is commonly called "Fruit Chats" in Indo-Pak subcontinent. They are frequently consumed in addition to mid day meal and are sold by street vendors at several public places in cities and towns. Although, appetizing and delicious, like all street food items, they are also exposed to abundant sources of contamination depending on the conditions in which they are sold, therefore, significantly contribute to food borne ailments (Kumar et al., 2006).

Several ailments caused by bacterial pathogens are cured by using different groups of antibiotics that are special class of chemotherapeutic agents obtained from living organisms. However, development of resistance in almost all bacterial species to different classes of antibiotics poses a major and global healthcare problem. Several mechanisms are known to induce antibiotic resistance in bacteria, but the most common type of resistance develops and transmits horizontally via conjugation of a plasmid. Likewise, evolution of multidrug-resistant (MDR) in bacterial strains, known as "superbugs" may create serious threat which results in resistance to several antibiotics (Alanis, 2005). Under these conditions, the treatment of such illnesses becomes difficult. However, the knowledge of susceptibility or resistance of bacterial species to various antibiotics may help in the use of effective treatment.

Keeping in view the above situations, the current study was aimed to reveal the extent of prevalence of bacterial populations in fruit chat samples to ascertain their food safety and standards. The food samples were collected from vendors with diverse serving and selling practices. Multan region was selected for this study as it is the third biggest and highly populated city of the Punjab province in Pakistan. Moreover, the susceptibility patterns of various bacterial isolates from several samples were assessed against the most commonly prescribed antibiotics to suggest effective curative measures for food borne ailments.

## **MATERIALS AND METHODS**

### **Samples collection**

A total of 120 samples of fruit chats were collected from different

localities around Multan city (30°11' 44" N, 71°28' 31" E). Fruit chat vendors were divided into four groups; mobile fruit chat vendors using no covering; mobile fruit chat vendors with covering; stationary fruit chat vendors; stationary fruit chat vendors having refrigerators. Thirty samples were taken from each of these vendors. The basic selection criterion was at least 100 customers per day, served by these vendors. Samples collected in pre-sterilized zip-lock bags were placed in ice boxes and brought to the laboratory for bacterial analysis.

### **Isolation and identification of bacteria**

Twenty-five grams of each fruit chat samples was suspended in 225 ml of buffered peptone water (Difco Labs, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.). They were aseptically homogenized in a stomacher and 0.1 ml of different dilutions used and were transferred onto the surface of plate count agar (Difco, BD Diagnostic Systems, Sparks, MD, USA), followed by incubation at 37°C for 24 h for total aerobic plate counts (TPC) and results were expressed as colony forming unit (CFU)/g (AOAC, 1990).

### **Characterization of bacteria**

All bacterial isolates were identified and were Gram-stained initially, characterized biochemically and identified up to species level by performing standard tests. The isolation and enumeration of *S. aureus* was carried out onto Baird Parker Agar (BPA) containing egg yolk emulsion by pour plate method with Tellurite emulsion (0.1 g/l of MgCl<sub>2</sub> and 0.05% tween 80) to enhance the cellular activities. The media was then transferred to Petri-dishes followed by incubation at 37°C for 24 h until black colonies of staphylococci appeared. Duplicate plates were employed for each dilution and plates containing 30 to 300 colonies were selected for count.

After preliminary identification, different tests were carried out on staphylococci. Coagulase by Goldstein and Roberts (1982), DNase by MacFaddin (1985) and for oxidase, Cheesebrough (2005) was followed. Gram-negative bacteria of the family *Enterobacteriaceae* were appraised by using various media: Violet Red Bile Lactose (VRBL); MacConkey Agar and Eosin Methylene Blue agar (EBM) and confirmed with API 24E Kits (Microbact 2000, Oxide Hampshire, England).

### **Disc diffusion susceptibility testing**

Antibiotics susceptibility patterns of various isolates of Gram-positive and Gram-negative bacteria were studied against different antimicrobial agents by disc diffusion method (Bauer et al., 1966). National Committee for Clinical Laboratory Standards criteria were used to interpret diameter of inhibition zone with specified potencies (NCCLS, 1991).

The disc diffusion for Gram-positive and Gram-negative bacteria was performed on Mueller Hinton Agar (CM0337-Oxoid) prepared according to manufacturer's instruction and sterilized by autoclaving at 121°C. Well isolated colonies (4 to 5) of the same morphological types were selected from an agar plate and transferred to a tube containing 4 to 5 ml of sterilized Mueller Hinton Broth. This broth culture was incubated at 35 ± 2°C until it achieved the turbidity of 0.5% McFarland standards. After adjusting the turbidity of inoculum suspension, a sterile swab was dipped into the adjusted suspension. The swab was rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab.

This procedure was repeated twice and plates were rotated 60° each time, to ensure even distribution of inoculum. Discs were

**Table 1.** Percentage total plate count, total coliform count and prevalence of various bacterial species in fruit chat samples collected from different street vendors.

Vendors	I	II	III	IV
TPC (Total plate count)	95.00±6.93	91.00±5.32	90.00±5.10	88.00±4.44
TCC (Total coliform count)	80.00±3.89	79.00±3.76	78.00±3.82	76.00±3.69
<b>Bacterial species</b>				
<i>S. aureus</i>	76.00±3.67d y	66.66±4.89c y	65.56±4.87b z	61.1±5.34a y
<i>S. epidermidis</i>	34.78±2.68d w	25.00±2.11a v	30.00±2.84b w	33.20±3.29c x
<i>E. coli</i>	39.10±2.52d x	37.5±2.34c x	35.00± 3.08b y	33.30±2.22a x
<i>Enterobacter</i> species	32.60±4.80d v	29.16±2.22c w	20.45±1.99b v	11.11±0.88a v
<i>Klebsiella</i> species	39.13±3.02d w	29.16±2.65b w	32.46±3.20c x	22.22±2.64a w
<i>Salmonella</i> species	21.70±2.76d u	20.83±1.98c u	15.24±1.34b u	05.55±0.23a u

I; mobile fruit chat vendors without any covering, II; mobile fruit chat vendors with covering, III; stationary fruit chat vendors IV; fruit chat vendors having refrigerators. Values are the Means ( $\pm$  S.E) from n number of samples (n = 30). Means sharing same letters across rows (for vendors) and columns (for bacterial species) are not significantly different by DMRT (Duncan, 1955) at 5% level of probability where LSD = 0.39 and 0.44 for vendors and bacterial species, respectively.

placed evenly (not closer than 24 mm from centre to centre) on the surface of the agar plate by using a sterile forceps. Twelve discs were placed on one 150 mm plate or 5 discs on a 75 mm plate. These plates were inverted and placed in an incubator at  $35 \pm 2^\circ\text{C}$  for 16 to 18 h within 15 min after placing the discs. Each plate was examined the next day and zone diameter was measured and incorporated as susceptible, intermediate or resistant (Bauer et al., 1996; NCCLS, 1991).

#### Data analysis

Data for bacterial prevalence were presented as percentages. Similarly, antibiotics susceptibility for each species is also presented as cumulative percentages. All percentages were arcsin transformed (Bliss, 1937). MINITAB version 15 was used for statistical analysis of the data. A two-way analysis of variance was carried out to elucidate significant differences between fruit chat vendors and bacterial species. DMRT (Duncan's Multiple Range Test) was used for multiple comparisons at 5% level of probability following Duncan (1955). In order to determine variability for antibiotics, bacterial species and susceptibility patterns, a General Linear Model (GLM) ANOVA was applied.

## RESULTS

### Bacterial prevalence

Percentage data for total plate count (TPC) and total

coliform count (TCC) in fruit chat samples collected from different vendors (Table 1) clearly indicated a high degree of contamination by a variety of bacterial species. *S. aureus* had consistently the highest percentage (95 to 88%) for TPC in samples from all vendors. However, *Salmonella* species had shown lower percentage (21 to 5%) prevalence. The other bacterial species (*E. coli*, *Enterobacter*, *Klebsiella*, *S. epidermidis*) had considerably lower percentage prevalence in samples obtained from different vendors. The percentage data (Table 1) regarding TCC also indicated a high degree of contamination in all samples but the total percentage for TCC was comparatively lower (80 to 76%) than TPC (95 to 88%).

Fruit chat samples obtained from mobile vendors without any covering were more contaminated (Table 2). The highest number of samples (15) indicated a bacterial count of  $10^7$  to  $10^8$  CFU/g followed by,  $10^6$  to  $10^7$  CFU/g from 10 samples. The level of contamination predominated in samples collected from mobile vendors without any covering and a higher total bacterial count ( $10^9$  to  $10^{10}$  CFU/g) is evident from the data (Table 2).

Likewise, TCC also showed similar ranges. Fruit chat samples collected from mobile vendors with covering also indicated contamination (Table 2). However, the extent of contamination was considerably low as TPC ranged

**Table 2.** Bacterial prevalence (CFU/g) of fruit chat samples collected from different street vendors in Multan, Pakistan.

	Bacterial range (CFU/g)						
	$10^3-10^4$	$10^4-10^5$	$10^5-10^6$	$10^6-10^7$	$10^7-10^8$	$10^8-10^9$	$10^9-10^{10}$
<b>Mobile vendors without covering</b>							
Total plate count	-	-	6	10	15	9	4
Total coliform count	-	-	4	9	15	7	2
<i>E. coli</i>	-	1	2	6	4	5	-
<b>Mobile vendors with covering</b>							
Total plate count	-	1	9	6	6	-	-
Total coliform count	-	1	8	5	5	-	-
<i>E. coli</i>	-	4	2	3	-	-	-
<b>Stationary vendors</b>							
Total plate count	-	3	5	5	5	-	-
Total coliform count	-	3	5	4	3	-	-
<i>E. coli</i>	1	3	-	3	-	-	-
<b>Stationary vendors with refrigerators</b>							
Total plate count	-	4	5	5	2	-	-
Total coliform count	-	4	3	5	2	-	-
<i>E. coli</i>	1	2	2	1	-	-	-

CFU = colony forming unit, - not detected.

**Table 3.** Analysis of variance for bacterial prevalence in fruit chat samples collected from different vendors; A; in Multan city; B; for susceptibility patterns of bacterial species against commonly used antibiotics.

Source of Variation	Sum of squares	df	Mean squares	Significance
<b>A</b>				
Vendors	745.09	3	248.366	**
Bacterial species	16225.10	12	1352.091	***
<b>B</b>				
Antibiotics	265.02	5	53.0	**
Bacterial species	8.85	5	1.77	**
Susceptibility	69031.27	2	34515.64	*
Antibiotics × Bacterial species	16.04	25	6.44	**
Bacterial species × Susceptibility	622.14	10	62.21	**
Antibiotic × Susceptibility	16735.47	10	1673.55	**
Antibiotics × Bacterial species × Susceptibility	6542.38	50	130.85	**

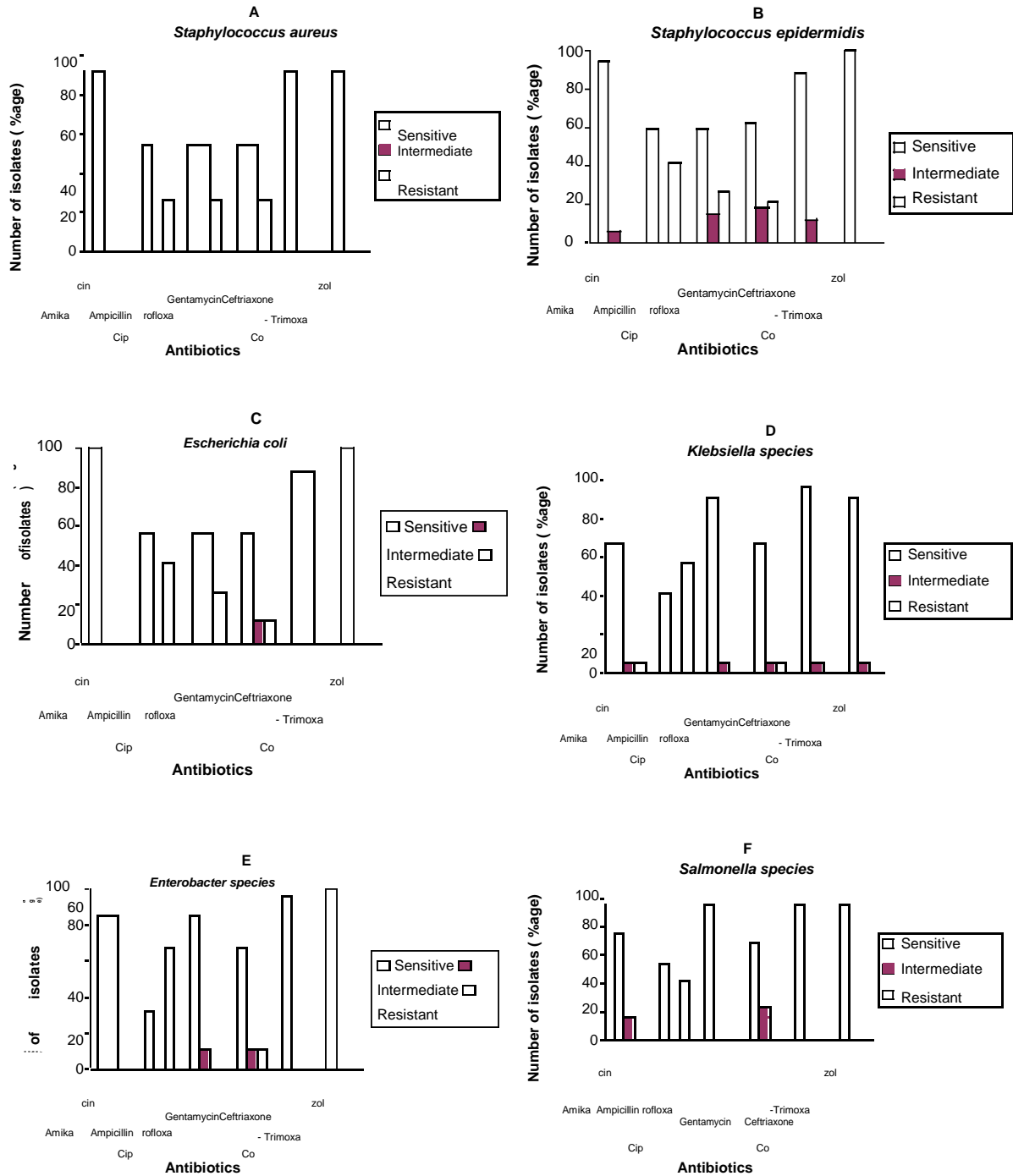
\*, \*\*, \*\*\* Significant at 0.05, 0.01 and 0.001% level of probability, respectively.

$10^5$  to  $10^6$  CFU/g from the highest number of samples as compared with samples from vendors without covering. The TCC also exhibited a similar trend and also ranged from  $10^5$  to  $10^6$  CFU/g.

The data also indicated a marked contrast for CFU/g with respect to the mobility of vendors as samples collected from stationary vendors had a lower bacterial count irrespective of refrigeration facility. The CFU/g value for TPC and TCC ranged from  $10^7$  to  $10^8$  in these samples.

Though, bacterial contamination was consistently observed in all samples collected from different vendors, the highest level of contamination was observed in samples collected from mobile vendors without covering. Overall bacterial contamination was considerably low in all samples which were collected from other vendors.

Analysis of variance of the data (Table 3A) revealed highly significant ( $P \leq 0.001$ ) variability among bacterial species and vendors. Multiple comparisons between Mean values (DMRT) depicted significant ( $P \leq 0.05$ )



**Figure 1.** Susceptibility patterns of various bacterial isolates; (A); *Staphylococcus aureus*; (B); *Staphylococcus epidermidis*; (C); *Escherichia coli*; (D); *Klebsiella* species; (E); *Enterobacter* species; (F); *Salmonella* species, from fruit chat samples against commonly used antibiotics.

variability for bacterial count in samples from mobile vendors without covering which differed markedly from all other vendors. Similarly, Mean values for all bacterial species also exhibited a marked contrast ( $P \leq 0.05$ ) except *Klebsiella* and *S. epidermidis* from vendors without covering (Table 1). Bacterial count was also

significantly different in samples from all other vendors but prevalence of *Enterobacter* and *Klebsiella* species did not show any significant dissimilarity in samples from vendors with covering.

The data for antibiotics susceptibility against six bacterial species is presented in Figure 1. The responses

of bacterial species isolated from fruit chat samples against various antibiotics depicted differential susceptibility pattern. However, all bacterial species had shown a greater sensitivity (more than 90%) against amikacin, ceftriaxone and co-trimoxazol, but *Klebsiella* species had no such response for amikacin and showed a comparatively lower (67%) sensitivity (Figure 1D).

The responses for ampicillin were consistent for all species as they had shown resistance for this antibiotic. The maximum resistance (67%) against ampicillin was observed in *Enterobacter* species (Figure 1E) followed by *Klebsiella* species (58%). In *S. aureus*, the lowest (33%) resistance was observed for ampicillin.

Gram-positive bacteria; *S. aureus* (Figure 1A) and *S. epidermidis* (Figure 1B) and Gram-negative *E. coli* and *Enterobacter* exhibited similar responses for ciprofloxacin and gentamycin where these species had different degrees of both susceptibility and resistance.

*Enterobacter*, *Klebsiella*, and *Salmonella* species were comparable for gentamycin only. However, two later species had also shown a similarity between them as both had shown some resistance for amikacin (Figure 1F). Amikacin had shown the maximum efficacy against *E. coli* (Figure 1C). Similarly, co-trimaxazol was found to be effective against all bacteria except *Klebsiella* species (Figure 1d) which depicted an intermediate resistance for this antibiotic.

ANOVA (Table 3b) depicted significant variability for antibiotics, bacterial species and susceptibility patterns ( $P \leq 0.01$  for all). The interaction was also found to be significant ( $P \leq 0.01$ ) for all main factors studied (antibiotics, bacterial species and susceptibility).

## DISCUSSION

### Bacterial prevalence

The results of the study clearly indicated considerable levels of contamination in fruit chat samples. The occurrence of bacterial species in fruit chat samples is parallel with several other studies which also indicated similar bacterial flora in same sort of food products (Houang et al., 1991; Beuchat, 1996). The source of these bacterial species may be soil, air, water and contaminated utensils. Francis et al. (1999) have reported the incidence of *S. aureus* in fresh produce and the level varied between 7 to 24% but this study indicated a high profile of this species (76 to 61%). The greater incidence of *S. aureus* can be attributed to the reason that this pathogen is known to be carried out by food handlers.

Members of the family *Enterobacteriaceae* have been considered a potent cause of food borne outbreaks (Centinkaya et al., 2008) but pathogens of this group did not show much prevalence. Similarly, *Salmonella* species reported here are in lower ranges than those reported for

various fruits and vegetables from different parts of the world (Beuchat, 1998).

The study demonstrated high ( $10^9$  to  $10^{10}$  CFU/g) total plate count and total coliforms. These bacterial count/ranges are comparable to Viswanathan and Kaur (2001) who worked out for bacterial prevalence in a similar street vended food item. Likewise, Christison et al. (2008) also reported high bacterial prevalence in filled baguettes and salads. These, and our study, clearly suggested reduced food safety of street vended fruits chats and salads which virtually remains the same, owing to the prevalent vending cultures and practices in most parts of the developing world.

Bacterial count was significantly higher in samples obtained from mobile vendors which do not use cover. Thus, these vendors appeared to pose a greater threat to consumers' health because of existed contamination levels. However, the bacterial prevalence seemed to be influenced by the presence of covering. Reduced bacterial contamination in fruit chat samples obtained from mobile vendors using covering clearly suggested that, covering to some extent act as a shield for air borne bacterial pathogens. However, higher degree of contamination in samples from other vendors could be attributed to substandard cutting and preparation practices, particularly poor hygienic conditions, of the premises that may result from, rubbish, sewage and other noxious substances present in the vicinity (WHO, 2010).

A comparatively lower bacterial count from samples collected from vendors with refrigeration facility clearly signified that keeping foods at relatively lower temperature, that is, below 8°C may increase food safety as compared to its storage at room temperature even for a short duration (WHO, 2006). Thus, our study validated that the use of refrigerator can reduce contamination to a large extent as compared to those vendors selling these products either mobile or stationary irrespective of a covering. Hence, bacterial counts could be further minimized to a significant level by refrigerating the raw materials or blended products, coupled with other sanitation and hygienic practices.

### Susceptibility patterns of bacterial species against different antibiotics

Since different fruits are available all round the year, therefore, a large population persistently consumes fruit chat in Multan city. High level of contamination of this food item is a potential hazard to consumers' health. Therefore, study was further extended to evaluate antibacterial activity of a range of commonly used antibiotics against prevalent bacterial species in fruit chat samples. As such, the results of the study could serve as ready reference to combat possible food borne outbreak.

The results of the study showed multiple antibiotic resistance in *E. coli*. A high level of resistance was

against ampicillin (42%) while low resistance (26%) was observed for gentamycin. The bacterial species had an intermediate sensitivity up to 10% for ceftriaxone. Developing resistance against ampicillin and gentamycin indicates a relatively reduced efficacy of these antibiotics. The resistant bacteria may colonize human intestinal tract and may also contribute resistant genes to existing endogenous bacterial flora. The transmission of enteric bacteria to consumers via food stuff is well established (Francis et al., 1999), therefore, food hygiene is of great concern to avoid food poisoning.

When considering the sensitivity of the bacterium, amikacin, ceftriaxone and co-trimoxazol appeared to be effective against *E. coli*. Thus, different susceptibility patterns against various antibiotics were established from this study and such responses had widely been reported for *E. coli* isolated from fresh products of vegetables and fruits (Kapperud et al., 1995; Olayemi, 1997; Busani et al., 2004; Centinkaya et al., 2008).

*Salmonella* species are the most important pathogenic bacteria implicated in food borne outbreaks (Centinkaya et al., 2008). The maximum resistance was noted for ampicillin (42%). The species also showed developing resistance for amikacin and gentamycin suggesting that these antibiotics can be least effective against *Salmonella* in the years to come. Viswanathan and Kaur (2001) and Farzana et al. (2009) reported that most of the isolates of *Salmonella* had higher degree of resistance to ampicillin. However, the species are sensitive to ciprofloxacin, ceftriaxone and co-trimoxazol. Though, resistance to antibiotics involves different mechanisms, greater sensitivity of *Salmonella* species for ciprofloxacin can be ascribed to an important property of drug as it inhibits the activity of enzymes that are involved in DNA replication such as topoisomerases and gyrases. Thus, DNA synthesis and in turn, colony formation is greatly inhibited (Galan and Curtiss, 1990). Ceftriaxone is also found to be effective against the bacterial species. Since the drug belongs to a third generation cephalosporin, hence, it emerged as an effective antibiotic and at present, it is frequently prescribed by physicians. Moreover, the efficacy of co-trimoxazol can be ascribed to its multidrug nature (Trimethoprim and sulphamethoxazole).

As far as the antibiotic resistance of Gram-positive bacteria (*S. aureus* and *S. epidermididis*) is concerned, it is evident from the results that both Gram-positive bacteria have similar resistance patterns for ampicillin, ciprofloxacin and gentamycin. Whereas, *Klebsiella* species (Gram-negative) had shown resistance for amikacin and gentamycin only. The antibacterial activity of amikacin, ceftriaxone and co-trimoxazol was greater for both Gram-positive species. However, the two later antibiotics have also shown their effectiveness for a Gram-negative species (*Klebsiella*) in addition to ciprofloxacin. Thus, ceftriaxone and co-trimoxazol can be drugs of choice for Gram-positive as well as for Gram-negative bacteria.

However, greater susceptibility of *S. aureus* and

*S. epidermididis* for amikacin is possibly due to its minimal use. In addition, the mode of action of ciprofloxacin is known to cause sensitivity in a number of bacterial species including *Klebsiella* species (Galan and Curtiss, 1990).

## Conclusion

The present study revealed bacterial contamination in street-vended fruit chats. Handling practices carried out by street vendors and the prevailing conditions in which these products are displayed is the main cause of contamination. The levels of contamination clearly suggested the potential risk of food borne out-breaks. Since, no international or local standards exist in the country, standardization of health and safety status of such products must be arbitrated by appropriate regulatory authorities which can minimize the risk of bacterial diseases to a great extent.

The study also indicated the development of multi drug resistance against commonly used antibiotics in bacteria isolated from, fruit chat samples. The development of multi-drug resistant in common pathogenic bacteria is of great health concerns all over the world because it makes the treatment of food borne outbreaks more difficult, particularly in developing countries like Pakistan, which already experiences poor health and hygiene conditions. The mounting resistance against antibiotic can be attributed to their constant use which poses strong selection pressure thus, favoring the evolution of resistance in bacterial species.

Based on the findings of this study, it can safely be concluded that preventative measures, particularly hygienic conditions, during the preparation of food stuff should strictly be improved. Additionally, in case of food borne outbreak, effective antibiotics should be used to treat infections and to avoid mounting resistance in pathogenic bacteria.

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