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Research Article

TO CHECK THE ANTIBACTERIALRESISTIVITY OF PATHOGENIC BACTERIA ASSOCIATED WITH COOKED AND UNCOOKED VEGETABLES, LAHORE, PAKISTAN

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ABSTRACT

Fresh vegetables normally carry natural nonpathogenic epiphytic microorganisms but during the growth, harvest, transportation and other handling produce can be contaminated with pathogens from animals and human sources. Most of these products are eating without further processing, their microbial content may be represent a risk factor for the consumer health and therefore a food safety problems. Safety of food is a basic requirement of food quality. A total of 50 different street food and vegetables samples randomly purchased from ten different vendors in different market. Seven bacteria were identified from the vegetables samples which belong to different genera, namely*: S. aureus, S. epidermidis, P. aeruginosa, P. putida, S.enterica, Shigellaspp*and *K. pneumoniae.* The analysis was done on carrots, potato, capsicum, bitter gourd, cabbage, brinjal, butternut squash, zucchini, lady finger, eddoes by using the different media. *S. enterica* (72%) were the most predominant bacterial isolates associated with vegetables, followed by *K. pneumoniae* (70%), *P. putida*(54%), *S. epidermidis* (46%), *S. aureus* (44%), *P. aeruginosa* (44%), while *Shigellaspp* (28%) was least predominant. The finding exposed that street foods are potential transportations for transmitting food borne illness thus the need to develop practical strategies geared toward street food safety.

INTRODUCTION

Vegetables are significant self-pride solid food and extremely good as mainstreamed of wellness and anticipation of disease. These are the fresh and edible piece of herbaceous plant which can be eaten raw cooked (Dhellot, 2006). Green leafy vegetables are luxurious sources of nutrients for growth in man and animal particularly in agrarian regions whereas he contributes significantly to macromolecule, materials, aliments, characters former nutrient while are often in abruptly furnish in everyday dieting (Mohammed and Sharif, 2011). The linked The inner tissue of healthy plants are free of microorganism, however the surfaces of raw vegetables are dirty with a mixture of microorganism and this depends on handling the time and condition of storage (Pelczar *et al.,* 2006).

The inner tissues of vegetables are nutrient rich and a PH near impartiality because the principal storage polymer is starch. Vegetables comprise mixture of therapeutic agents. Vegetables can also regarded as the comestible constituent, such basic leaves, stalks, roots tubers, bulbs, plants and seeds. Now a day, new vegetables juices are significant diet in different cities of the world. They serve as an authentic and reasonable therapeutic benefits extensively better which encourage cleansing of human (Deanna and Jeffrey, 2007).

Show the possible sources of bacteria e.g*E. coli*, species of Salmonella, Shigella, and Staphylococcus etc. Most of the outbreaks have been related with microbial pollution. Vegetables normally carry a nonpathogenic epiphytic micro flora (Ray, 2004). Uncooked vegetables port a number of pathogenic viruses, which may be discrete over the plant or appear as micro gatherings surrounded in the plant tissues (Beuchat, 2002). During the harvesting and transport, uncooked vegetables may be injured resulting in the announcement of plant nutrient, if substrates for microorganism present on the superficial of the vegetables to produce. The dispensation of fresh salad vegetables may adapt or increase the number and types of pathogens present on the surface of the creation. Human food is that spent to uphold life and development of body (Ezeronye, 2007).

Microbial food decay is any vicissitudes which make the food to be unbearable for consumption. Spoilage occurs due to indecorous handling, cooking, cooling, and needless time lag between preparation and consumption (FDA, 2007; Munide and Kurai, 2005). The unhygienic handling of food ,health

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status of food sellers, poor condition of souks and nonappearance of drivable water disposal facilities ,use of dirty water and poor storing affect food safety (WHO, 2001-2003;Ghosh*et al.*, 2007 and Kamil, 2005).

Consumption of street food has grown over the years due to the fast population growth, unemployment, poverty and availability of relatively low cost foods (Chakravarty and Canet, 2002). Symptoms of food borne illness are diarrhea, queasiness, stomach cramp and biliousness (Nweze, 2010). It may be caused by *Staphylococcus aureus*, Salmonella app, Entro pathogenic *E. coli*. Street food is food drink that it could be raw or cooked. The dissimilar diversities of street food in Pakistan evolve round the communal starchy clips of vegetables like tomatoes, onions, green pepper, lettuce, cabbage, incentives and cucumber. In Pakistan mixed salad are ready with fresh lettuce, tomato, onion carrots or green pepper (Johnson and Yawson, 2000).

The danger of disease upsurge through the vegetables dirty by ill water varies from a region to added, because the bacterial and parasitic broadcast has adjustable gradation in the dissimilar region (Steele, M. and J. Odumeru, 2004). Scientific reports have exposed that the outbreaks of food borne increasing, chiefly with renewed vegetables. These are numerous intelligences that uncooked vegetables are the pathogenic porter. Salmonella and *E. coli* have unglued from uncooked vegetables. These microorganisms may soil the vegetables in the establishing, reaping, Storing and issuing stages. Some spates due to ingesting fresh spuds are stated every year Mukherjee*et al*., 2006).

Vegetables cover valued food fixings cover. Studies have assessed the group of vegetable ingesting exact. Cooked food can be branded as the collection of nourishment being prepared for instant ingesting; it could be uncooked or cooked and can be hooked without further action. Food borne disease is an ever current threat that can be prevented with banned with proper care and treatment of food product. It is projected that between 24 and 81 million case of food tolerated diarrhea illness that $17 billion in medicinal care is lost in output (MacPherson and Tonkin, 2004). Bacteria food killing is the most shared type of food murdering and it is produced as a result of the company of damaging bacteria or toxic material shaped by them in food. An eruption of food killing may be produced by food which seems to be quite dissimilar from those complicated in food decay (Okonko *et al*., 2008).

Staphylococcus aureus a Gram optimistic catalase positive coccus that contain cells that seem as a grapes shape when we see below microscope. The connection between the *Staphylococcus aureus* and food killing has been associated with consumption cheese. *Staphylococcus aureus* is also related with enterotoxin arbitrated food killing. Consumption of foods dirty with *S. aureus* can cause gastroenteritis, nausea, vomiting, diarrhea and stomach pain within 1-6 hours post ingesting of dirty foods (EFSA, 2007). The process of culinary should kill the microorganisms but some bad does of handling or storing can even increase the microbial load of the first product (Tivadar, 2003). Most of the food killing are a result of insanitary actions and unsuitable handling does by human (Adams and Moss, 2003). Pathogens can be approved and approved on the others by persons who themselves are not ill. Such transporters may have lately hurt about of food killing and still be protecting the creature in their body. In some cases transporters of food killing act as host over a retro of many years having themselves acquired protection to organisms worried e.g.,*Salmonella typhi*, *Staphylococcus aureus*(Nichol and Salek, 2007).

Thus the objective of the present work were isolation, identification, characterization and antimicrobial sensitivity of different bacteria isolated from uncooked and cooked vegetables collected from various localities of Lahore, Pakistan.

MATERIALS AND METHODS

Source of samples

Samples were collected from five different locations, Wapda town, Valencia town, Gulshane Lahore society, Food Street of Valencia town and home.

Collection and processing of sample

Fifty samples, twenty five uncooked samples, five each of carrot, potato, capsicum, bitter gourd, cabbage and twenty five cooked samples potato(4), brinjal(3), capsicum(2), butternut squash(4), zucchini(5), lady finger(4), eddoes(2). Cooked and uncooked vegetables samples were collected from each location and packed into plastic containers, and transport into laboratory and processed immediately to prevent corrosion. 25g sample was aseptically weighed and 150ml of sterile distilled water was added and blended it.

Isolation of microorganism

Six fold serial dilutions were then prepared by transferring one ml of the original well mixed sample, 1:6 dilutions to a tube containing 9ml of sterile physiological saline. Each test tube was labeled with the type of sample and number of the dilution. Sample was inoculated on the nutrient agar plate through swabbing, and incubated on 37°C for 24 hours, then bacterial colony was streak on nutrient agar for purification and on differential and selective media such as mannitol salt agar, eosine methylene blue agar, macConkey agar, salmonella shigella agar, cetramide agar, blood agar for the identification of bacteria.Gram staining and biochemical tests were performed for the identification and confirmation of bacterial isolates.

Antibacterial Susceptibility Test (AST)

Antibacterial Susceptibility testing was done by Kirby-Bauer method of disc diffusion, modified by CLSI (Clinical Laboratory Standard Institute) technique on Mueller-Hinton agar(Bauer *et al.*,1966) using antibiotic discs corresponding to the drugs most commonly used in the treatment of human and animal infections caused by bacteria; All isolates were selected for antibiotics sensitivity test according to Kirby-Bauer method by using only 10 types of antibiotics disc; Cefotaxime (CTX 30mcg), Tobramycin (TOB 10mcg), Ciprofloxaxin (CIP 5mcg), Meropenem (MEM 10mcg), Imipenem (IPM 10mcg), Ceftazidime (CAZ 30mcg), Amikacin (AK 30mcg), Aztreonam (ATM 30mcg), Levofloxacin (LEV 5mcg), Cefepime (FEP 30mcg) for bacterial isolates were used for determining the

antibacterial and the zone of inhibition was measured (Willy *et al*., 2008; Cappucino and Sherman, 2007).

RESULTS

Total 50 vegetables were process to check the microbial contamination. Out 50 sample, different cooked and uncooked sample of vegetables from different towns of Lahore which consist of carrot, capsicum, bitter guard, potato, brinjal, cabbage, lady finger, Butternut Squash, Eddoes, zucchini, butternut. From these vegetables 7 different bacterial isolates were obtained which are *S. aureus, S. epidermidis, P. aeruginosa, P. putida, S. enterica, Shigellaspp, K. pneumoniae.* In Figure 1 the Number of bacterial isolates from different sources. This shows that *K. pneumoniae* is present in large quantity from food street samples. *P. putida* is present in small quantity from Gulshan e Lahore and home samples.

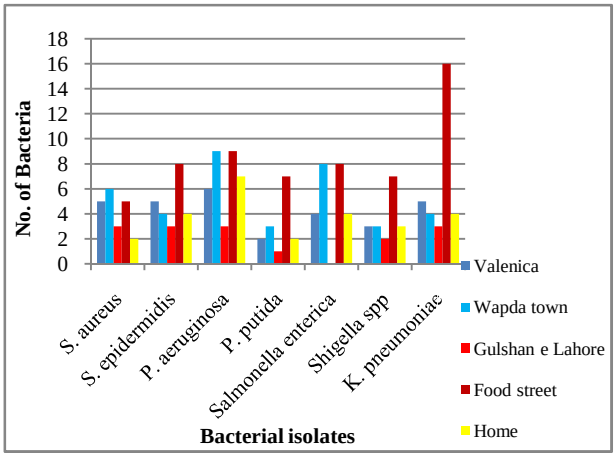

Fig 1 Isolation of bacterial isolates from different location of Lahore

In figure 2 shows the percentage of bacterial isolates. 70% *S. enterica* is present in all samples and 25% Shigellaspp is present in all samples.

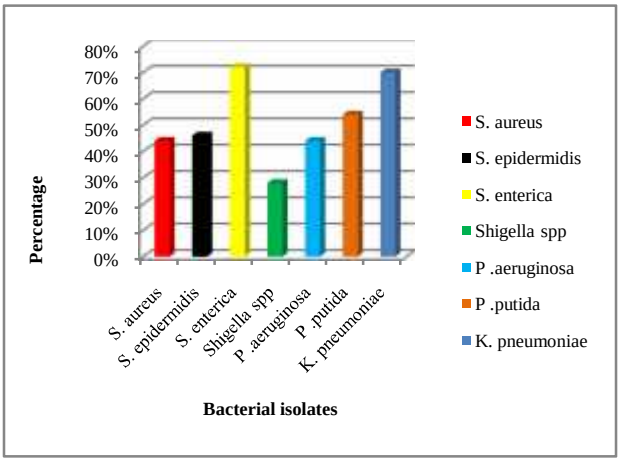

Fig 2 Percentage of bacterial isolates from different vegetables

Find out the separate percentage of bacterial isolates which is associated with the uncooked vegetables and cooked vegetables to checkout their difference. This figure shows the percentage of cooked and uncooked vegetable samples of their compression. 72% *K. pneumoniae* is present in cooked samples and 64% *K. pneumoniae* is present in the uncooked samples. 24% *P. putida*is present in the uncooked samples.

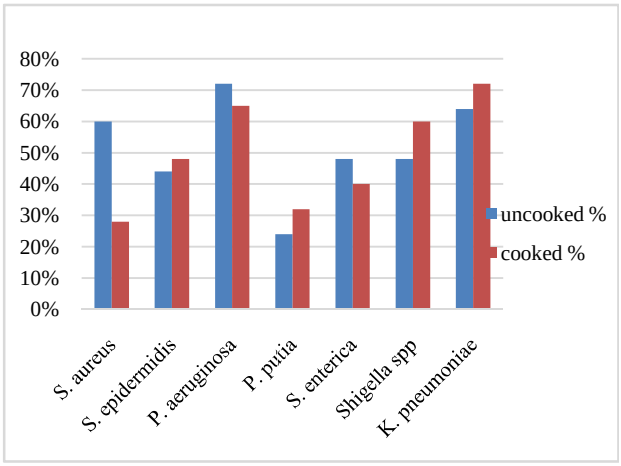

Fig 3 Percentage occurrence of Bacterial isolates associated with the uncooked and cooked vegetables

Biochemical test was performed for the conformation of bacterial isolates. Indole test, methyl red, citrate test, oxidase test, urease test, H_2S and TSI test, catalase and coagulase test all these test was performed for the conformation of bacteria. The (+) sign shows the positive results and (-) sign shows the negative results. Y* shows the yellow color, B** shows the black color and R*** shows the red color.

Table 1 Biochemical test of Bacteria associated with the vegetables

Sr.No	Bacterial isolates	Indole	Methyl red	Citrate	Oxidase	Urease	H_2S	TSI	Catalase	Coagulasge
1.	*S. aureus*	_	+	_	_	_	_	_	+	+
2.	*S. epidermidis*	_	+	_	_	_	_	_	+	_
3.	*P.aeruginosa*	_	_	+	+	_	_	Y/R	+	_
4.	*P. putida*	_	_	+	+	_	_	Y/B	+	_
5.	*S. enterica*	_	+	+	_	_	+	R/B+	+	_
6.	*Shigella spp.*	+	+	_	_	_	_	Y/B/-	+	_
7.	*K. pneumoniae*	_	+	+	_	+	_	Y/Y/gas	+	_

In Table-2 CTX shows 100% resistivity against all bacterial isolates. While CIP, MEM, IPE, AK, FEP shows the 0% resistivity. MEM, IPE, FEP shows the 100% sensitivity, while CTX shows the 0% sensitivity.

Table 2 Sensitivity and resistivity of Bacterial isolates

Abbreviation	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*P. putida*	*S. enterica*	*Shigella spp.*	*K. pneumoniae*	*Sensitivity*	*Intermediate*	*Resistivity*
CTX	R	R	R	R	R	R	R	0	0	100
TOB	R	S	S	R	R	S	R	43	0	57
CIP	S	S	S	I	1	S	I	57	43	0
MEM	S	S	S	S	S	S	S	100	0	0
IPE	S	S	S	S	S	S	S	100	0	0
CAZ	R	R	R	R	R	S	R	14	0	86
AK	S	S	I	S	I	S	S	71	29	0
ATM	S	S	S	R	R	S	R	57	0	43
LEV	S	S	S	S	R	S	S	86	0	14
FEP	S	S	S	S	S	S	S	100	0	0
Sensitivity	70	80	70	50	30	100	50			
Intermediate	0	0	10	10	20	0	10			
Resistivity	30	20	20	40	50	0	40			

DISCUSSION

Vegetables are significant self-pride solid food and extremely good as mainstreamed of wellness and anticipation of disease. These are the fresh and edible piece of herbaceous plant which can be eaten raw cooked. Green leafy vegetables are luxurious sources of nutrients for growth in man and animal particularly in agrarian regions whereas contributes significantly to macromolecule, materials, aliments, characters former nutrient while are often in abruptly furnish in everyday dieting.

The high microbial weight obtained in urban farm could be directly linked to the recorded greater waste water used in irrigation that could be from sewage water for watering the field or the use of manure used for fertilization and the insanitary condition of the area where the vegetables were being grown. The slight difference in the microbial weight from other sources can be traced to the prewashing and refreshing water. Among the organisms encountered during this study *S. aureus* showed the highest incidence in all the five locations. It reported that ingestion of the thermos enterotoxins, rather than the bacterium itself is accountable for foodborne illness (Mead *et al*., 1999).

Common symptoms of Staphylococcal intoxication contain nausea, vomiting, retching, abdominal cramping, sweating, chills, prostration, weak pulse, shock, shallow, respiration, and subnormal body temperature. *S. aureus* is commonly originate in the nose and throat and on the hair and skin of more than 50% of healthy individual any food like vegetables that are frequently handled may therefore easily contaminated (Tivadar, 2003) highlighted the of increasing prevalence of eating away from home and the use of partly or fully cooked food. Most of the vendors who sold both raw and cooked food were not regulated; they operated haphazardly without any monitoring of what they prepared and how they prepared it (Abdalla *et al*., 2008a).

P. aeruginosa disease of vegetables it has become an important cause of infection and it is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections and bacteremia (Aloush *et al.*, 2006). Salmonella is a common name applied to a group of nearly 2000 biochemical linked serotypes conscientious for food borne illness. The disease is hideously underreported because it is normally self-limiting gastroenteritis which may be misdiagnosed as intestinal influenza by patient or the physician. As an outcome estimates of the true occurrence of disease are based as assumptions derived from epidemiological evidence. Clearly, Salmonellosis continues to be an important cause of food borne disease worldwide (FDA, 2004).

Shigella are transmitted during the feacal matter of people or animals and are typically transmitted through the faecal matter of people or animals and are typically transmitted to humans by eating foods that have been contaminated with faecal matter through cross contamination. Generally, food borne shigellosis is characterized by a high attack rate, common cause epidemiology. The problems recognized to bacterial infection have enlarged significantly, in terms of both incidence and severity. Furthermore, an increase of antimicrobial resistance in this pathogen makes the treatment of infection more difficult that perhaps results in death. Therefore, epidemiological information and monitoring systems are necessary to control bacterial infection in public health division. In this study, we observed frequency and antimicrobial susceptibility patterns of bacterial isolated from animal protein, chicken meat, and vegetables. The study showed that the marketing animal protein was highly contaminated by different bacteria, followed by cooked and fresh vegetables. These products, according to many reports, are the resources of Salmonella contamination. In addition, it had also been reported the sub-therapeutic measures of antimicrobial drugs in animal husbandry as a responsible feature in appearance and maintenance of multiple antimicrobial resistant pathogenic bacteria. According to several reports, our results confirmed that the fluoroquinolone groups such as ciprofloxacin and norfloxacin are still the most effective drugs to treat bacterialinfection. In recent years, indication for decreasing exposure to fluoroquinolones in different bacteriahas been reported. Increasing resistance to fluoroquinolones is growing as an issue receiving special Attention, since fluoroquinolones are effective drugs against different bacteriain clinical performance and are usually considered as treatment of choice in life threatening cases.

This study found that bacteria isolated from fresh vegetables and cooked vegetables samples and antimicrobial resistance patterns.*S. enteric* (72%) were the most predominant bacterial isolates associated with vegetables. This was followed by *K. pneumoniae* (70%), *P. putida* (54%), *S. epidermidis* (46%), *S. aureus* (44%), *P. aeruginosa* (44%), while Shigellaspp (28%) was least predominant. More than one pathogenic microorganism was isolated from vegetable samples. The finding exposed that street foods are potential transportations for transmitting food borne illness. Improper food handling practices, poor hygienic condition of places where vegetables were displayed use of contaminated equipment's and containers during transportation contributed to contamination of these pathogenic microorganisms.

This suggests that contamination in vegetable samples may originate from environmental sources that are different from animal contamination. However, this study indicated that retail vegetables can serve as a source for MDR strains of bacterial isolates that may transfer to vegetables. Association or cross contamination between vegetable samples is subject to further evaluations.

CONCLUSION

It was concluded that seven bacterial isolates viz. *S,entercia, K. pneumoniae, P. aeruginosa, P. putida, S. aureus, S. epidermidis*was isolated from the spoiled food samples.Street food business has remained principally unfettered in Pakistan. Wholesome and nutritious street foods have a positive impact on food security, while utilization of street foods The finding of this study demonstrate that bacterial contamination is present in carrot, potato, capsicum, bitter gourd, cabbage, butternut squash, zucchini, lady finger, eddoes sold in different markets of Lahore. Salmonella spp is high, since it is more significant and can cause food poisoning. Other organisms isolated such as *S. aureus,S. epidermidis, P. aeruginosa, P. putida*, Shigellaspp and *K. pneumoniae* which were also isolated in insignificant number could still cause food borne illness depending on the consumer's health status. *P. putida* is less significant in

vegetables samples. Therefore, it is very important and essential for food vendors to always clean and sanitize food contact surface, cook and store food properly, so as to reduce the level of food contamination and also to reduce bacterial load to the lowest, thereby preventing cases of food borne infections. Factors such, as the vendors personal cleanness, the type of food, have an effect on the bacterial contamination present in foods.

Recommendations

Cleaning preventing irritated contamination is both essential in making sure that the food served is safe to eat. Effective cleaning gets liberate of bacteria on hands, equipment and surface which help to stop injurious organisms from spreading into food. Vendors should make sure that

- Clean food surface and equipment, especially after handling raw foods.
- Maintain raw food and already to eat foods separate.
- Cook food appropriately and store food in a safe place away from insects.
- Wash utensils and surfaces before and after use with hot, soapy water. But still, sanitize with diluted bleach one tea spoon of bleach to one quart of hot water.

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