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Determination and Identification of *Enterobacteriaceae* in Street Vended Foods in Karachi, Pakistan

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Abstract: Food borne diseases are an increasingly recognized problem involving a wide spectrum of illnesses caused by bacterial contamination of food. *Enterobacteriaceae* poses potential human health problems and is mainly transmitted through consumption of contaminated foods. Karachi is one of the biggest and thickly populated city of Pakistan where majority of the people consume cheap foods prepared in unlicensed food selling points. The objective of the present study was to determine the total *Enterobacteriaceae* load in various fast foods sold directly to consumers in the streets of Karachi. A total of 27 different food items were collected from street vended foods and processed for the recovery of *Enterobacteriaceae* load through culturing method and biochemical characterization. Out of the 27 samples, 13 food samples did not show the growth of *Enterobacteriaceae*, while the remaining 14 samples were heavily contaminated with gram negative bacteria. From the present study, it can be concluded that majority of the fast foods sold in the street restaurants of Karachi Pakistan is highly contaminated and not fit for human consumption.

Key words: Microbial contamination, fast foods, unhygienic conditions

INTRODUCTION

Pakistan comes under developing country with the current population in 2013 exceeding 180 million (WPS, 2013). The annual rate of urbanization is 3.1% with a current 36% of total population living in urban areas (PDP, 2013). The urban people are at a high risk of food and water borne diseases due to poor sanitary practices. Karachi is one of the biggest trading and thickly populated metropolitan city of Pakistan with an estimated 23.5 million population in 2013. Street food is a wide variety of ready to eat foods and beverages prepared in public places and sold relatively cheaper and easily accessible. Food safety assurance is poorly exercised in this city and there are hundreds of fast food cabins which un-hygienically prepare and sell cheaper food with a potential risk of microbial transmission in the human food chain. The consumption of street foods is common in Karachi due to high rate of unemployment, low wages, limited social awareness program and high population. The street foods are well appreciated by consumers, due to its taste, low cost, nutrients value and availability for immediate consumption (Ghosh *et al*., 2007). In the context of poverty, street food account for a part of the family income, daily diet and so contribute towards meeting nutritional requirements. However, the unhygienic conditions in which these foods are prepared, stored and served raise a question regarding their microbiological quality. Thus, street food importance has consequences such as its association to epidemic and disease outbreak in case of microbiological quality failure (Barro *et al*., 2006; Estrada-Garcia *et al*., 2004). Food is contaminated through poor food handling and unhygienic procedures. The *Enterobacteriaceae* is a large family of gram-negative bacteria that includes, more familiar pathogens, such as *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella* and *Yersinia*. Most of the members of *Enterobacteriaceae* cause gastrointestinal problems in human beings (Paterson, 2006). The diseases are caused by either toxin from the disease-causing microbes, or by the human body's reactions to the microbe itself (Teplitski *et al*., 2009). The disease is sometimes difficult to treat due to the emergence and spread of resistance in *Enterobacteriaceae* (Paterson, 2006). Traditional media culturing and biochemical characterization of *Enterobacteriaceae* are still an authentic widely used methods for isolation and identification of bacteria from many sources including consumable foods (Bohaychuk *et al*., 2005; Glynn *et al*., 2006; Worcman-Barninka *et al*., 2001).

Our hypothesis that most of the street vended food sold in Karachi city is highly unhygienic and thus may carry heavy load of potential human pathogenic gram negative bacteria, was tested by collecting food samples from the

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street vended food shops and processed for the recovery of total *Enterobacteriaceae* load. The isolated bacteria were biochemically characterized into species.

MATERIALS AND METHODS

Samples collection: Street vended food samples (one from each food item) were collected from different fast food shops in Karachi, Pakistan. The samples were from; strawberry shake, mango shake, chaat, pakoray, dahi baday, shish kebab, beef kabab, french fries, chicken biryani, beef biryani, salad, shashlik, fried rice, golgappy, ice-cream, jalebi, fruit cake, sweet biscuit, chutney, white tea, patties, samosa filling, chicken burger, raw chicken, aachar, curry and kachoori. From each of the above mentioned items, the required amount was poured into 20 mL sterile containers and brought to the Microbiology Laboratory of University of Karachi for further processing. Samples were processed on the day of collection.

Samples preparation and culturing on media: From each sample, 1 mL or 1 g was added into 9 mL phosphate buffered saline (PBS) and mixed thoroughly. A 100 µL aliquot of PBS was plated on Violet Red Bile Glucose Agar (VRBGA, Oxoid UK), incubated overnight at 37°C and examined for colony growth of *Enterobacteriaceae*, if any. The total number of colonies on each VRBGA plate was counted using an ordinary magnifying glass. A control plate was incubated in the same procedure in order to check the sterility of the VRBGA plates used.

Isolation and purification of *Enterobacteriaceae*: From each incubated plates showing different characteristics colonies, a single colony was then isolated and grown overnight at 37°C in VRBGA. Standard dilutions were prepared by dissolving the grown colony in 20 mL PBS and matching its turbidity with 0.5 McFarland standards (bioMerieux France). Enumeration of viable bacteria was performed by serial dilution and plating 100 µL of each solution on VRBGA plates and incubated overnight at 37°C. The cultural characteristics of each isolated colony were examined visually and each pure colony was further processed for biochemical characteristics of *Enterobacteriaceae*.

Biochemical characterization of the isolates: Colonies were presumptively identified by colony pigmentation and Gram staining characteristics. Triple sugar iron (TSI) and IMViC tests were used to identify the individual bacteria isolated from food samples. In acronym "IMViC", "I" is for indole test; "M" is for methyl red test; "V" is for Voges-Proskauer test and "C" is for citrate test. These tests are useful in distinguishing members of *Enterobacteriaceae*. For the TSI procedure, individual colonies were inoculated into the TSI tubes and incubated overnight at 37°C. Next day, reaction (color) in Slant and Butt was recorded in addition to gas and H_2S production. Similarly, for the IMViC tests, single colony of individual bacteria was inoculated into respective media, incubated overnight at 37°C and read the reaction.

Table 1: Gram negative bacterial load in food samples

Food sample	10 log CFU/mL of food	Food sample	10 log CFU/mL of food
Strawberry shake	2.60	Beef biryani	2.90
Mango shake	2.70	Salad	2.30
Fruit chaat	2.68	Ice-cream	1.85
Pakoray	2.56	Jalebi	1.60
Dahi baday	2.86	Samosa filling	1.90
Shish kebab	1.60	Chicken burger	2.00
Beef kebab	2.78	Raw chicken	2.72

Data analysis: For the bacterial load determination in 1 mL of food samples, the number of colonies were counted by using a magnifying glass and converted into 10 log CFU (colony forming unit). The TSI and IMVIC results were recorded as acidic, alkaline, positive or negative.

RESULTS

Among the total of 27 menu items, gram negative bacteria were identified in 14 food samples as shown in Table 1. Beef biryani showed high level of bacterial load followed by dahi baday and beef kebabs respectively. Bacteria was not isolated from food samples of; French fries; chicken biryani, shashlik, fried rice, gol gappay, fruit cake, chutney, white tea, aachar, curry and kachori. The number of 10 log CFU in 1 mL of positive food sample is shown in Table 1. The most heavily contaminated food was beef biryani with a 2.90 10 log CFU/mL of food. The isolated bacteria were; *Salmonella*, *Serratia fanticola*, *Klebsiella pneumonia*, *Escherichia coli*, *Shigella dysentriae*, *Pseudomonas aeroginosa*, *Acinetobacter*, *Proteus mirabilis* and *Providencia rettgeri*. There was no bacterial growth on the control plates, which showed the absence of cross contamination during the whole procedure.

The bacteria isolated in the study showed the typical colony characteristics which are explained in Table 2.

The result of the biochemical characterization test is shown in Table 3. These tests confirmed the specie of bacteria isolated from the food items.

DISCUSSION

As we hypothesized, there was a heavy load of gram negative bacteria in the street vended fast foods sell daily in the streets of Karachi city. In the current study, all the major food items which are consumed on daily basis had higher *Enterobacteriaceae* load. The highest bacterial load in per gram of food item indicates that these foods pose a greater threat to the consumer's health (Glynn *et al*., 2006). Interestingly, all the food

Table 2: Colony characteristics of gram negative bacteria isolated from food samples

Isolate	Colony characteristics
Salmonella	Lac-, irregular margin, opaque, white, medium sized flat colony
Serratia fanticola	Lac+, dark pink, variable in size and shape, flat, medium sized colony
Klebsiella pneumonia	Lac+, light pink, large, round, dark centered, translucent and convex colony
Escherichia coli	Lac+, medium sized, white large center pink colony, round, convex colony
Shigella dysentriae	Lac-, opaque, irregular, white, convex colony
Pseudomonas aeroginosa	Lac-, large, transparent and flat colony
Acinetobacter	Lac+, dark pink, medium sized, light centered and raised colony
Proteus mirabilis	Lac+, off white center pink watery, round and convex colony
Providencia rettgeri	Lac+, irregular, medium sized, light pink, creamy, round, small and flat colony

Lac-: lactose non fermenting bacteria; Lac+: lactose fermenting bacteria

Table 3: Isolates characteristics determined by TSI and IMViC tests

	TSI							
Isolate	BUTT	SLANT	Gas	H_2S	IND	MR	VP	Citrate
Salmonella	Acidic	Acidic	+ve	+ve	-ve	+ve	-ve	-ve
Serratia fanticola	Acidic	Acidic	-ve	-ve	-ve	+ve	-ve	+ve
Klebsiella pneumonia	Acidic	Alkaline	+ve	-ve	-ve	-ve	+ve	+ve
Escherichia coli	Acidic	Acidic	+ve	-ve	+ve	+ve	-ve	-ve
Shigella dysentriae	Acidic	Acidic	-ve	-ve	-ve	+ve	-ve	-ve
Pseudomonas aeroginosa	No change	No change	-ve	-ve	-ve	-ve	-ve	+ve
Acinetobacter	No change	Alkaline	-ve	-ve	-ve	-ve	-ve	-ve
Proteus mirabilis	Acidic	Acidic	-ve	-ve	+ve	+ve	-ve	+ve
Providencia rettgeri	Acidic	Acidic	-ve	-ve	+ve	+ve	-ve	+ve

items having highest bacterial load is favourable foods for the consumers not only in Pakistan, but also in many other countries. Gram negative bacteria could not isolated from the thirteen food items which might explain that these food items were processed more hygienically during its preparation or the processing procedures of their preparations killed bacteria. During food preparations, heat above 60°C for at least 15 min can significantly kill microbes (Gogus, 2012). It can also be assumed that some of the food items negative for *Enterobacteriaceae* might have antibacterial molecules which kill bacteria, but this needs to be further tested. Some phenolic compounds present in various foods have antimicrobial activity against gram negative bacteria (Medina *et al*., 2007). A high pH of some foods is another factor that kills bacteria (Mendonca *et al*., 1994).

The presence of heavy load of gram negative bacteria in food showed the poor quality of the food which further put question mark on its wholesomeness. The presence of *Enterobacteriaceae* reflects the existence of favourable conditions in respective food items for the growth of microorganisms. Further, the results reveal that most of the sellers don't process and handle foods hygienically. The gram negative bacteria isolated in this study are pathogenic and the contaminated food could be of high risk in transmitting enteric pathogens in the human food chain interaction. Food poisoning in the city is mostly linked due to consumption of unhealthy foods. A number of factors like unhygienic surroundings, contaminated water, improper waste disposal system, use of single water bucket for washing all the dishes, no hand washing facilities, unhygienic preparation and processing of foods might be the possible sources of food contamination in these sites. Almost all the bacteria isolated in this study are pathogenic to human at various degrees and infections caused by some bacteria are even hard to treat with increase in the rate of antibiotic resistance in the community (Fernandez-Fuentes *et al*., 2014).

Conclusions: It can be concluded that most of the street vended fast foods sold in the streets of Karachi city is highly contaminated with potential enteropathogens and is thus not fit for human consumption. Further investigation is needed to compare the difference of the contamination level of street vended food and standard restaurants food in the city and the level of antimicrobial resistance of the isolates to commonly prescribed antibiotics by the health professionals.

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REFERENCES

Barro, N., A.R. Bello, A. Savadogo, C.A.A. Ouattara, A.J. Ilboudo and A.S. Traore, 2006. Hygienic status assessment of dishwater, utensils, hands and pieces of money in street foods vending sites in Ouagadouguo; Burkina Faso. Afr. J. Biotechnol., 5: 1107-1112.

Bohaychuk, V.M., G.E. Gensler, R.K. King, J.T. Wu and L.M. McMullen, 2005. Evaluation of detection methods for screening meat and poultry products for the presence of foodborne pathogens. J. Food Prot., 68: 2637-2647.

Estrada-Garcia, T., C. Lopez-Sancedo, B. Zamarripa-Ayala, M.R. Thompson, L. Gutierrez-Cogco, A. Mancera-Martinez and A. Escobar-Gutierrez, 2004. Prevalence of Escherichia coli and *Salmonella* spp in street-vended food of open markets (tianguis) and general hygienic and trading practices in Mexico city. Epidemiol. Infect., 132: 1181-1184.

Fernandez-Fuentes, M.A., O.M. Elena, A. Hikmate, P.P. Ruben and G. Antonio, 2014. Antimicrobial resistance determinants in antibiotic and biocide-resistant gram-negative bacteria from organic foods. Food Control, 37: 9-14.

Ghosh, M., S. Wahi, M. Kumar and A. Ganguli, 2007. Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella spp*. in some raw street vended Indian foods. Int. J. Environ. Health Res., 17: 151-156.

Gogus, U., 2012. Effects of pressurization on some contamination flora in beef pate. J. Food Sci., 77: M550-M559.

Glynn, B., S. Lahiff, M. Wernecke, T. Barry, T.J. Smith and M. Maher, 2006. Current and emerging molecular diagnostic technologies applicable to bacterial food safety. Int. J. Dairy Technol., 59: 126-139.

Medina, E., C. Romero, M. Brenes and A. De Castro, 2007. Antimicrobial activity of olive oil, vinegar and various beverages against foodborne pathogens. J. Food Prot., 70: 1194-1199.

Mendonca, A.F., T.L. Amoroso and S.J. Knabel, 1994. Destruction of gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. Appl. Environ. Microbiol., 60: 4009-4014.

Paterson, D.L., 2006. Resistance in gram-negative bacteria: *Enterobacteriaceae*. Am. J. Infect. Control 34: S20-S28.

Pakistan Demographic Profile (PDP), 2013. Online retrieved from: http://www.indexmundi.com/pakistan/demographics_profile.html.

WPS 2013. World population statistics. online retreived from: http://www.worldpopulationstatistics.com/karachi-population-2013/

Teplitski, M., A.C. Wrigh and G. Lorca, 2009. Biological approaches for controlling shellfish-associated pathogens. Curr. Opin. Biotechnol., 20: 185-190.

Worcman-Barninka, D., M.T. Destro, S.A. Fernandes and M. Landgraf, 2001. Evaluation of motility enrichment on modified semi-solid Rappaport-Vassiliadis medium (MSRV) for the detection of *Salmonella* in foods. Int. J. Food Microbiol., 64: 387-393.