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...
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, golgappay, 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 H2S 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.

**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 kachoori. 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, 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...
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 be 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 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).

**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.

**ACKNOWLEDGEMENTS**

We are thankful to Dr. Shahana Urooj Kazmi, Chairperson of Microbiology Department, University of Karachi, who helped us in designing the experiment and manuscript.

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