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***Research Article** (Open Access)*

ASSESSMENT OF MICROBIAL CONTAMINATION OF FOOD SOLD IN THE BEACHES OF CHENNAI, INDIA

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ABSTRACT
Food is the most important item in the list of goods and services consumed by urban consumers, accounting for approximately 55 percent of total household expenditure. Most of the food is often bought and consumed outside home. These foods are sold by the food vendors and traders. Microbial contamination is a major food safety concern because it can cause a variety of foodborne illnesses. Many foodborne illnesses and outbreaks involving the contamination of food products with pathogenic bacteria, viruses and protozoa have been reported, and such outbreaks are being investigated and sources identified. Foodborne diseases are the major challenge to public health worldwide; this has greatly increased the cost of the health burden, especially in developed societies where time is money and it is very important to manage as human beings try to survive and prosper while fulfilling their needs. The microbiological quality of food samples sold in the beaches of the city of Chennai, India was evaluated and was found to be poor. In this study, the dominant fungus recovered from the food samples was *Aspergillus niger* (36%), followed by *Mucor spp* (24%), *Rhizopus spp* and *Penicillum spp* (12%), *Aspergillus flavus* and *Aspergillus fumigatus* (8%). All the food samples showed the presence of yeast. *Bacillus spp* (56%) was the leading bacteria isolated from the food samples, followed by *Escherichia coli* (20%), *Staphylococcus aureus* and *Klebsiella pneumoniae* (8%), and *Pseudomonas aeruginosa* (4%). All of these pathogenic microorganisms can be a potential cause of foodborne illness.

Keywords: *street food, bacterial pathogens, fungal pathogens, foodborne pathogens, Chennai*

INTRODUCTION
Microbial spoilage is a major problem for so-called perishable foods such as fresh fruits, vegetables, meat, poultry, fish, baked foods, milk and juices. The factors influencing the growth of food microbes and the ever-evolving community also determine the nature of contamination and the health risks that may arise due to convenience. Internal food factors include nutrients, growth factors and inhibitors, water activity, pH and oxidation reduction potential. Effect of each factor on growth in a food system, factors co-occur and affect microbial growth together, either favorably or negatively (Ray and Bhunia, 2013).
The food prepared and/or sold by vendors in public places are valued for its unique taste and convenience and for maintaining the nutritional value of traditional foods. Street food ensures food security for poor urban dwellers. Vendors often lack food hygiene, work in harsh and unsanitary conditions, and have little or no knowledge of the causes of foodborne illness. Despite its health benefits, people consume street food in their daily life, which is sold on streets, public places, busy markets, school premises, university campuses, near taxi stands, beaches, etc. Although there is little research on street food in India, some studies have shown that as much as 20-30% of Indian food is consumed as street food (Kharel *et al*., 2016).
Foodborne diseases are a major problem today, as many diseases are caused by bacterial, viral, parasitic or chemical contamination of food. In addition, the resistance of these microorganisms to several drugs has made this situation a public health concern. Diarrhea is the most common cause of food poisoning in humans and can lead to death in some cases. Diseases are caused either by the poison produced by the microorganism or by the reactions of the human body against the microorganism (Tabashsum *et al*., 2013).
Food-borne pathogens are extremely harmful to human health because they enter the human digestive tract

immediately after food consumption. A significant portion of prepared food is sold as street food and is typically consumed in low-profile socio-economically developing countries. Ready-to-eat foods, including cut fruits, lettuce, sprouts and vegetables, are known for their impact on foodborne outbreaks in both developed and developing countries. Various food materials are sold under unsanitary conditions, especially in developing countries, causing various foodborne illnesses to consumers. Outbreaks of human infection have increased due to the consumption of such foods, despite their nutritional and health benefits (Saleem *et al*., 2014).

Foodborne pathogens occur in a variety of foods. Their detection is important to ensure a safe food supply and to reduce foodborne illnesses, as they are the most important health problems worldwide. Over the past three decades, the scientific community has faced major safety challenges, including new foodborne pathogen strains, adulterated foods, long-term consumption of genetically modified foods, presence of chemicals in foods, and food nutrition. A large number of people around the world suffer from diseases (dysentery, food poisoning and diarrhea) directly caused by various food-borne pathogens such as *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Shigella spp* and *Salmonella spp*. In addition, food-borne microorganisms (such as *Acinetobacter spp*, *Pseudomonas spp*, *Botrytis spp*) can cause huge amounts of food waste and lead to economic ruin by spoiling large quantities of food. Although they have not been implicated in any foodborne outbreaks, some of them may be opportunistic human pathogens (Hameed *et al*., 2018).

MATERIALS AND METHODS

SAMPLE COLLECTION AND PROCESSING

A total of 52 samples of food was collected from 4 different beaches of Chennai city namely Marina beach, Besant nagar beach, Kovalam beach and Tiruvanmiyur beach. The samples included in the study were those that could be consumed readily by the consumers. The samples were collected from stationary and mobile vendors near and away from the seashore and which can be consumed raw or after cooking. All the samples were collected in sterile containers and stored at 4^{o}C till they were processed.

ENUMERATION OF MICROORGANISMS

TOTAL AEROBIC COUNT

About 1 gram of the food samples were serially diluted in saline to obtain 10^{-1} to 10^{-8} dilutions. 0.1ml of each dilution was pipetted out and spread on to the Nutrient agar (NA) and Saboraud's Dextrose agar (SDA) plates and incubated for 24 hours at 37^{0}C for NA and 2 to 7 days at 25^{o}C for SDA plates respectively. Duplicate plates were maintained. After incubation, the number of colonies on the plates were counted and expressed as Cfu/gram, using the calculation as shown,

$$\text{Total aerobic count} = \frac{\text{Average no. of colonies}}{\text{Volume of the sample}} \times \text{Dilution factor [Cfu/g]}$$

ISOLATION OF BACTERIA

DIRECT EXAMINATION

MACROSCOPIC APPEARANCE: The macroscopic characteristics of different colonies on the Nutrient agar [NA] and Sabouraud dextrose agar [SDA] plates were observed and recorded.

CULTURE: Different colonies on NA were inoculated onto the following culture medias such as MacConkey agar (MA), Mannitol salt agar (MSA), Eosin methylene blue agar (EMB), Thiosulphate Citrate Bile Salt agar (TCBS), Cetrimide agar (CA) and Salmonella Shigella Agar (SS). The agar plates were incubated at 36± 1°C. The plates were examined after 24 hours and the colonies were tested by the following methods.

PRELIMINARY TESTS: Tests such as Gram stain, Hanging drop motility technique, Catalase test and Oxidase test were performed.

IDENTIFICATION OF BACTERIA

The colonies on the selective media were further processed for biochemical identification.

BIOCHEMICAL TESTS: All the biochemical tests were performed with suitable positive and negative controls (Mackie and McCartney, Practical Medical Microbiology, fourteenth edition 1996). The tests performed were Indole test, Methyl Red (MR) test, Voges Proskauer (VP) test, Citrate utilization test, Triple Sugar Iron (TSI)

test, Urease test, Nitrate reduction test, Oxidation and Fermentation (OF) test, DNase test and coagulase test..
SUGAR FERMENTATION TEST: This test was performed to detect the production of acid and gas on ferentation of sugars used such as glucose, fructose, lactose, sucrose and mannitol. Suitable controls were included in the test.

ISOLATION OF FUNGAL ISOLATES

DIRECT EXAMINATION

MACROSCOPIC APPEARANCE: The macroscopic characteristics of the different colonies on Sabouraud Dextrose Agar [SDA] plates were observed and recorded.
CULTURE: Each fungal colony was inoculated onto Sabouraud Dextrose Agar plates to obtain pure culture and for further identification. The plates were incubated at 25°C and were examined after 5 days and colonies were identified.
LACTOPHENOL COTTON BLUE STAINING: After macroscopic examination of the fungal culture carefully on Sabouraud Dextrose Agar plate, the colony was picked up with a sterile loop and placed on the LPCB stain. With the help of a tweezing needle, the specimen was tweezed well without disrupting the intact morphology of the fungus. A clean coverslip was placed over the specimen and excess of stain was also removed with the help of a filter paper and observed under 45X.

RESULTS AND DISCUSSION

A total of 52 food samples were collected from 4 different beaches of Chennai city, India were examined and the following results were obtained.

Total Aerobic Count

The average of the total aerobic microbial count of the food samples obtained from Nutrient agar (NA) and Sabouraud's dextrose agar (SDA) were summarized by taking an average count in Table 1 and 2.

Table 1: Total bacterial count in food samples

Sample Type	Total bacterial count		Average count Cfu/g
	1	2	
Chickpea Sundal	22.0×10^8	20.4×10^8	21.2×10^8
Bread Samosa	29.2×10^7	29.8×10^7	29.5×10^7
Panipuri	19.9×10^7	21.3×10^7	20.6×10^7
Bhel puri	15.4×10^7	15.0×10^7	15.8×10^7
Cotton candy	29.2×10^5	29.7×10^5	29.6×10^5
Masala puri	20.6×10^7	19.6×10^7	20.1×10^7
Sweet Corn	16.3×10^5	16.1×10^5	16.2×10^5
Soan papdi	17.9×10^5	18.7×10^5	18.2×10^5
Boiled ground nut	16.1×10^7	17.5×10^7	17.4×10^7
Raw mango	30.8×10^6	33.5×10^6	32.3×10^6
Plantain Bajji	18.3×10^5	18.6×10^5	18.3×10^5
Boiled Corn masala	28.2×10^5	29.8×10^5	29.5×10^5
Pav bhaji	32.0×10^6	30.4×10^6	31.4×10^6

Table 2: Total fungal count in food samples

Sample Type	Total Fungal Count		Average count Cfu/g
	1	2	
Chickpea Sundal	39 x 10^4	34 x 10^4	30 x10^4
Bread Samosa	22 x 10^2	17 x 10^2	22 x 10^2
Panipuri	63 x 10^2	40 x 10^2	10 x 10^2
Bhel puri	12 x 10^2	20 x 10^2	8 x 10^2
Cotton candy	52 x 10^4	7 x 10^4	14 x 10^4
Masala puri	25 x 10^4	27 x 10^4	21 x 10^4
Sweet Corn	22 x 10^4	13 x 10^4	17 x10^4
Soan papdi	28 x 10^4	27 x10^4	26 x 10^4
Boiled ground nut	37 x 10^4	36 x 10^4	35.5 x 10^4
Raw mango	15 x 10^4	15 x 10^4	14 x 10^4
Plantain Bajji	25 x 10^2	22 x 10^2	22 x 10^2
Boiled Corn masala	13 x 10^4	11 x 10^4	12 x 10^4
Pav bhaji	35 x 10^2	32 x 10^4	23.3 x 10^4

The present study indicated that most of the samples collected from 4 different beaches of Chennai were contaminated and numerous potential sources of contamination were identified. Almost all the samples analyzed were found to be of poor microbiological quality. The total bacterial count in the present study revealed a maximum load of 21.2x10^8 cfu/g in chickpea sundal. The least count was observed as16.2x10^5 cfu/g in sweet corn. The counts of the present study were comparatively higher than the study, which reported high bacterial load in Spring Rolls and chowmein ranging from 10^5 to 10^6 cfu/mg amongst the fast food samples obtained near MMU Campus Mullana, Haryana, India (Dahiya *et al.*, 2018). Other samples like Bhel-puri and Pani-puri had relatively low bacterial counts in the range of 10^2 to 10^4 Cfu/ml.

Table 3: Percentage of fungal pathogens isolated

S.No	FUNGAL ISOLATES	PERCENTAGE PREVALANCE
1	*Yeast*	100
2	*Mucor spp*	24
3	*Aspergillus niger*	36
4	*Aspergillus flavus*	4
5	*Aspergillus fumigatus*	4
6	*Rhizopus spp*	12
7	*Penicillium spp*	12

The total fungal count in the present study revealed a maximum yeast load of 35.5 x10^4 cfu/g in boiled ground nut. The least count was observed as 8x10^2 cfu/ml in Bhel Puri. The total fungal count observed in another study, was significantly higher in snacks such as meat pie (21.4±2) while the least count was observed in buns (5.9±1.6) (Amadi *et al*., 2014). The reason behind such poor quality of food sold in beaches can be due to the presence of faecal contamination and pathogenic microorganisms in dry and wet sand.
The fungal pathogens isolated from various food samples with their percentage prevalence are shown in Table 3 and Figure 1.

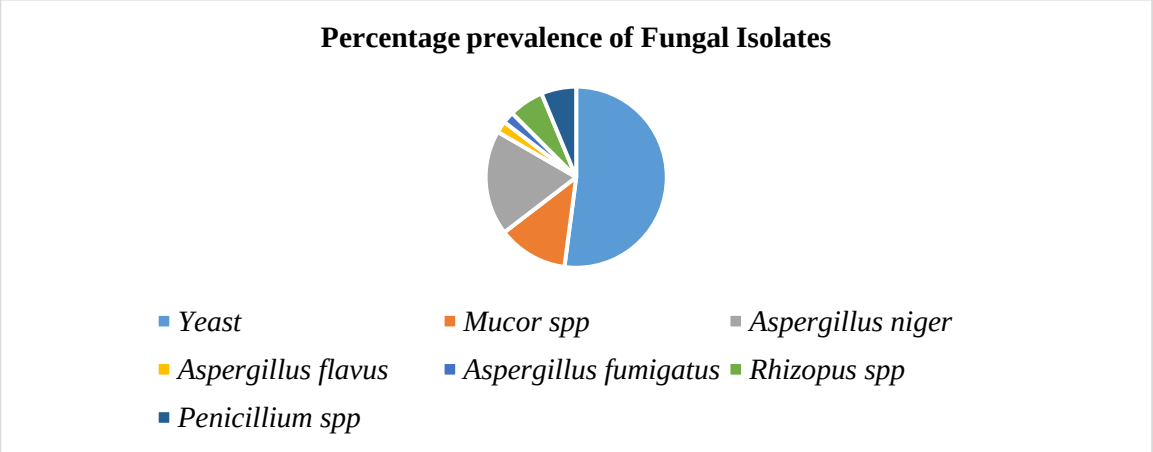

Figure 1: Percentage prevalence of fungal isolates in food samples

The bacterial pathogens isolated from various food samples with their percentage prevalence are shown in Table 4 and Figure 2.

Table 4: Percentage of bacterial pathogens isolated

S.No	BACTERIAL ISOLATES	PERCENTAGE PREVALENCE
1	*Bacillus species*	56
2	*Staphylococcus aureus*	8
3	*Escherichia coli*	20
4	*Klebsiella pneumoniae*	8
5	*Pseudomonas aeruginosa*	4

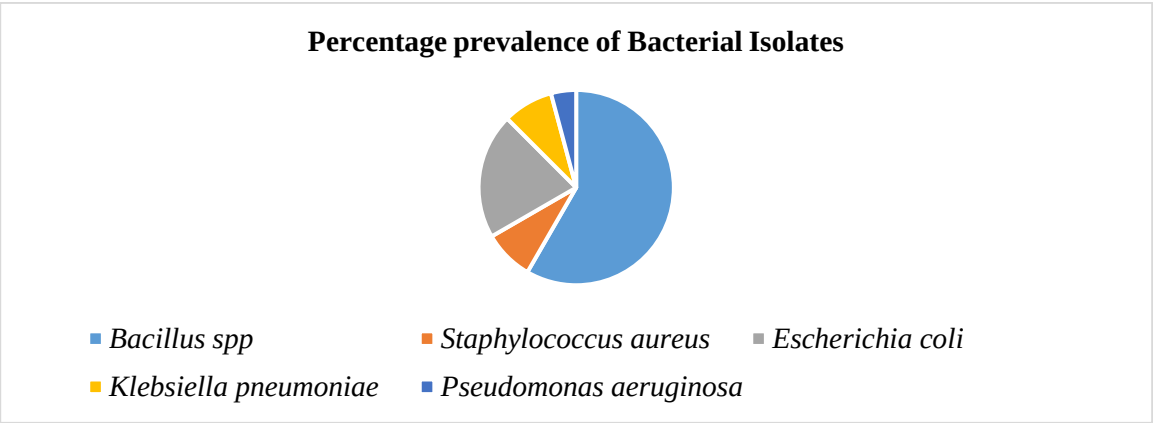

Figure 2: Percentage prevalence of bacterial isolates in food samples

In coastal areas, outbreaks of gastroenteritis, hepatitis, salmonellosis, viral illnesses and occurrence of dermatitis and mycosis have usually been associated with marine pollution. Recently these factors have also been correlated with the contamination of beach sands, resulting from wastes left by users and from solids deposited by tides (Mendes *et al*., 1993). *Vibrio parahaemolyticus*, *Salmonella spp* and yeast belonging to genus *Candida* were isolated from the major beaches in Brazil (Viera *et al*., 2001). The present study showed the presence of different fungi and this may be due to not wearing special uniform during working or not using appropriate methods and means to keep the equipment and the surrounding clean. Also, food processing is done in an open place close to beach and sand.

The dominant molds recorded in the snacks and chats in the present study belong to *Mucor spp*, *Aspergillus niger*, *Rhizopus spp* and *Penicillium spp*. *Aspergillus niger* (36%) is the dominant fungi in the present study followed by *Mucor spp* (24%), *Rhizopus spp* (12%), *Penicillium spp* (12%), *Aspergillus flavus* (4%) and *Aspergillus fumigatus* (4%) respectively. This is similar to the study, which revealed that *Asperigillus sp.* was the most spoilage fungi isolated from most type of foods (Anwar *et al*., 2017). The most frequent moulds encountered from fresh juices were *Aspergillus flavus*, *A. tereus*, *A. niger* and *Penicillium islandiarm* (Aneja *et al*., 2014).

The present study showed the presence of pathogenic bacteria such as *Bacillus spp* (56%) which was the predominant bacteria isolated followed by *Escherichia coli* (20%), *Staphylococcus aureus* (8%), *Klebsiella pneumoniae* (8%) and *Pseudomonas aeruginosa* (4%). *Bacillus cereus* is a group of ubiquitous facultative anerobic spore forming Gram-Positive rods commonly found in soil. The spores of *Bacillus* frequently contaminate a variety of foods, including produce, Meat, eggs, and dairy products (Tallent *et al*., 2012).

Staphylococcus aureus was isolated in high numbers and the most prevalent of the organisms isolated, however their study revealed contamination in the ready to eat snacks (Azounwu *et al*., 2018). The result obtained from their study showed that the snacks examined were all contaminated. But the present study isolated *Staphylococcus aureus* only from 8% of the samples analyzed. Staphylococcal food-borne disease (SED) is one of the most common causes of FBD worldwide. Outbreak Investigation has suggested that improper handling of cooked or processed food is the main source of contamination. Lack of cold chain allows *S. aureus* to form staphylococcal enterotoxins (SEs) (Kadariya *et al*., 2014).

Escherichia coli was isolated from 20% of the samples tested. The main source of *E. coli* contamination might be through contaminated water supplies used to hand and dish washing.The presence of *E. coli* and other coliform bacteria could be due to inadequate hand washing by food workers and poor processing practices (Tambekar *et al.*, 2009). *E. coli* contaminating the food is mainly due to unhygienic practices. *E. coli* were isolated in 30.7% of samples of different food items (Namita and Saxena, 2016).

Bacillus sp and *Klebsiella sp* were isolated from spring roll samples, *Escherichia coli* in chowmein sample and *Salmonella sp* in Dhal-puri and Pani puri samples. However, Burger and samosa samples were found to be contaminated with *Pseudomonas* and *Enterobacter sp* (Dahiya *et al*., 2018). The pathogenic bacteria such as *Escherichia coli* (18%), *Salmonella sp (15%)* and *Staphylococcus aureus* (7%) were isolated from ready to eat fast foods in Al-Quwayiyah, kingdom of Saudi Arabia (Alharbi *et al*., 2019).

Ready to eat food has been implicated in cases of food poisoning or gastroenteritis. The contamination of food products with infectious organisms and their persistent growth, multiplication and toxin production is an important public health concern. A study conducted on selected fried ready to eat food items revealed the presence of 21% of *B. subtilis*, 1.3% of *Streptococcus faecalis* and *Klebsiella sp* (Ochei Kingsley *et al*., 2014). The occurrence of *Pseudomonas aeruginosa* might be due to improper personal hygiene, unhygienic surroundings, vehicular activity, and proximity to sewage. The present study revealed the presence of *Pseudomonas aeruginosa* in 4% and *Klebsiella pneumoniae* from 8% of the tested food samples obtained from the beaches of Chennai.

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