

Screening of Street Vended Food for their Microbiological Quality and Antibiotic Susceptibility Testing of the Isolates

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ABSTRACT

Street food vending is a prevailing and distinctive part of a large sector in Ulhasnagar region. Street food is commonly consumed by a population of an age group ranging from 5-10 years to 60-70 years. This study was initiated with a view of determining bacterial agents associated with the street vended food and to investigating the antimicrobial resistance of the isolates. This study assessed the microbiological quality of various ready-to-eat unprocessed food sold in the Ulhasnagar region by street vendors. Microbiological analysis was conducted on 50 samples which included panipuri, vadapav, samosa, sambar, chutney, fruit juices, etc. The isolates were identified by growing them on selective media like MacConkey agar, SS agar, Mannitol Salt agar and performing various biochemical tests. Organisms isolated included: *Klebsiella* spp., *Serratia* spp., *Pantoea* spp. & *Staphylococcus aureus*. Interestingly *E. coli* was not isolated in any of the samples. As these food products are unprocessed and consumed directly, there are high chances of pathogenic microorganisms getting entry into the body and cause disease state. Kirby-Bauer disc diffusion method was performed to determine the sensitivity of the isolated microorganisms towards antibiotics. The isolates were resistant to antibiotics like Ceftazidime, Imipenem, and Tetracycline. Isolates showed an intermediate sensitivity to Gentamycin. This indicated that the vended street food is potential reservoir for the spread of emerging multiple drug resistant bacteria strains that pose threat to human health. This should draw the attention of relevant authorities to ensure that the hygiene standards are improved to curtail food born infections. Also it is necessary to educate the vendors to cut down the storage time, maintain proper cleanliness and use purified water for preparation of unprocessed food products.

Key words: Street vended food, Selective media, Kirby-Bauer method, Gastrointestinal tract infections.

INTRODUCTION

Diseases caused by consumption of food are increasing day-by-day, which is the major problem around the globe. The disease causing factor in food is majorly viral, bacterial and parasitic content. Also addition of adulterants

and chemical contaminants causes disease state condition. Mostly patients admitted in hospitals related to foodborne infections are thought to be majorly due to presence of bacterial agents in the food products.

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Diarrhoea is one of the most commonly caused diseased state in case of consumption of contaminated food. If untreated excessive loss of body fluids in diarrhoeal state may be fatal. The factors causing disease in the case of consumption of contaminated food are reaction of the microorganisms present in the food with individual's body or due to production of toxins by certain microorganisms⁴.

Street foods are ready to eat food products and liquid refreshments prepared by vendors and hawkers especially in an open area, and sold on streets and other similar public places².

The specific taste and flavour of the street food is the main reason for their increasing demand. Street food also serves as an income source for a considerable amount of population of developing countries. However due to lack of knowledge about health and hygiene between vendors, inappropriately prepared, stored and served food products have raised a question regarding their microbiological quality⁴.

Pathogenic microorganisms commonly detected in food products openly sold on the streets are, *Bacillus cereus*, *Pantoea agglomerans*, *Enterobacter spp.*, *Klebsiella spp.*, *Staphylococcus aureus* and *Salmonella spp.* Continuous consumption of contaminated street vended food products causes diseases like, diarrhoea, cholera, typhoid fever and food poisoning¹⁶.

Hence, subjecting the street vended food for microbiological analysis can help in identifying the pathogenic microorganisms associated with a particular food product. Continuous consumption of food products contaminated with a harmful pathogens can be lethal to an individual with an immunocompromised state. Determining susceptibility of microorganisms isolated from food products is also equally important. Depending upon the sensitivity of the microorganisms towards the antibiotics, they can be classified as sensitive or resistant strains. Multiple drug resistant bacteria is an

emerging strain which possess resistance to many antibiotics including third generation antibiotics. Spread of such multiple drug resistant bacteria through street vended food can cause threat to human life. Drug resistant strains once reach intestine, there can be horizontal gene transfer to intestinal flora. When these organisms are in contact with organisms in nature, it will lead to emergence of drug resistant strains in the natural environment.

Recently, rise in the antibiotic resistant microorganisms in humans have caused a major concern. The main reason for emergence of antibiotic resistant strain is application of inadequate therapeutic protocols. During the processing and preparation of food, due to improper methods and techniques followed often leads to contamination of the food by enteric pathogenic microorganisms⁵.

In India, large hawker centers are present in Delhi, Mumbai and Gujrat. The typical taste and flavour of the street food is greatly appreciated by the local as well as tourist people. Since street food is consumed by a considerable amount of population, it is necessary to take into the consideration about food safety. And efforts are to be made to ensure about sanitation and correct protocols are followed while preparation and storage of the street food products, so that people can consume street food products with confidence⁸.

MATERIAL AND METHODS

Food items All foods used in this study were purchased from various street vendor's outlets from the Ulhasnagar region of Thane district. Commercially available Nutrient agar, MacConkey's agar, MSA agar, Salmonella Shigella agar were used for isolation. Glucose phosphate broth, Simmon's citrate Agar, Tryptone broth, Triple sugar iron agar were used for performing biochemical tests. Mueller- Hinton agar was used for antibiotic susceptibility testing of the isolates¹.

The solid food products (like smashed potato i.e ragda, outer covering of samosa, potatovada etc.) were weighed 5 g each. Weighed sample was inoculated in 5 ml of sterile physiological saline in a centrifuge tube and was subjected to centrifugation at 1500 rpm for 4 minutes¹.

The liquid food products (like fruit juices, chutneys etc.) were taken 5 ml each in a

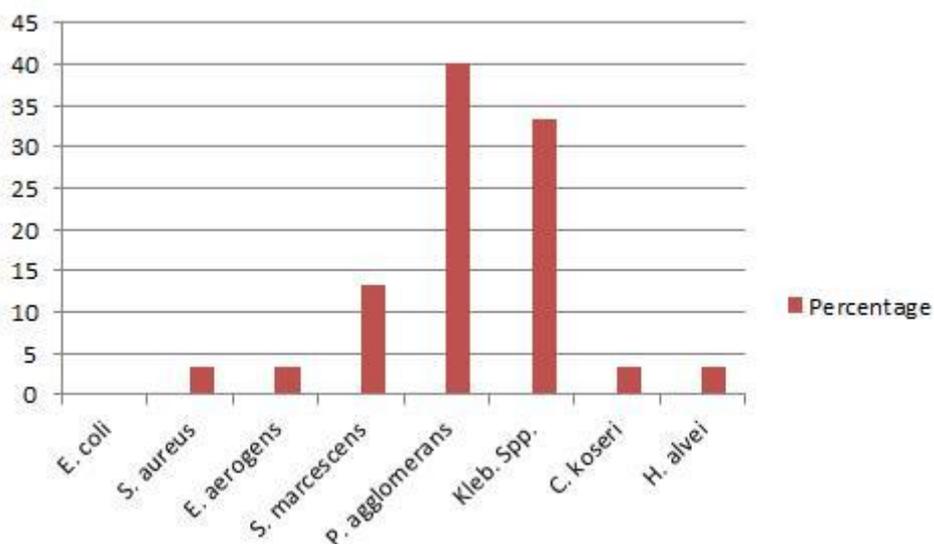
centrifuge tube and subjected to centrifugation at 1500 rpm for 4 minutes¹.

Isolation was performed on selective media. Specific colonies were selected in order to determine their colony characteristics and were subjected to Biochemical tests¹⁷.

The organisms identified from biochemical tests were then subjected to Antibiotic susceptibility testing (AST)¹⁸.

RESULTS

Graphical representation of Isolated microorganisms from food samples processed



Biochemical Tests:

Figure 01: Biochemical Tests



IMVIC and TSI Test

Table 1: Organisms isolated from different food samples

Samples processed	Number	Isolates obtained							
		<i>E. coli</i>	<i>S. a</i>	<i>E. aerogens</i>	<i>Serratia marcesens</i>	<i>Pantoea Agglomerans</i>	<i>Kleb. spp</i>	<i>C. koseri</i>	<i>H. alvei</i>
Pineapple Juice	02	0	0	1	1	0	0	0	0
Samosa	04	0	0	0		2	2	0	0
Chutney Schejwan	01	0	0	0	0	0	1	0	0
Sweet Chutney	05	0	0	0	2	1	1	1	0
Spicy Chutney	06	0	0	0	0	4	1	0	1
Uthappa	02	0	1	0	0	0	1	0	0
Sugarcane Juice	02	0	0	0	0	1	1	0	0
Chocolate milkshake	2	0	0	0	1	0	1	0	0
Sweet Lassi	1	0	0	0	0	1	0	0	0
Sambar	1	0	0	0	0	1	0	0	0
Potato Vada	4	0	0	0	0	2	2	0	0
Total	30	0	1	1	4	12	10	1	1
Percent	(100%)	0%	3.33%	3.33%	13.33%	40.00%	33.33%	3.3%	3.3%

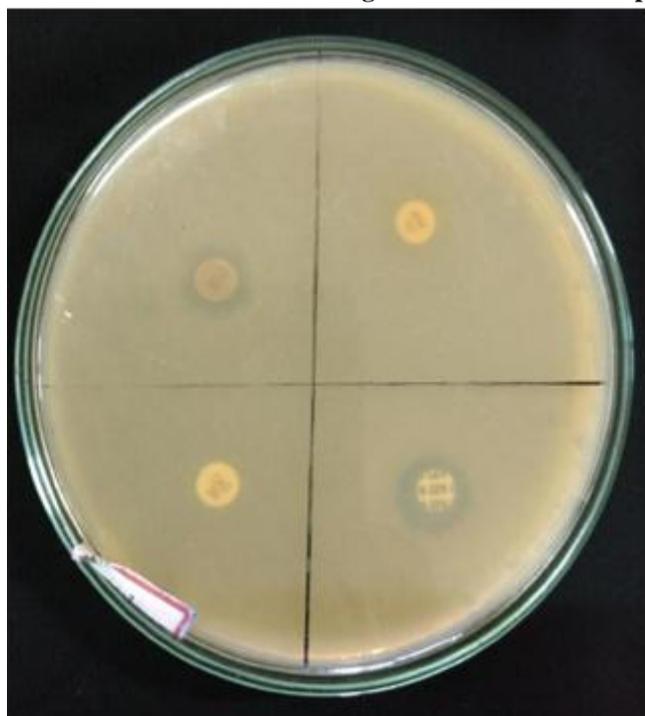
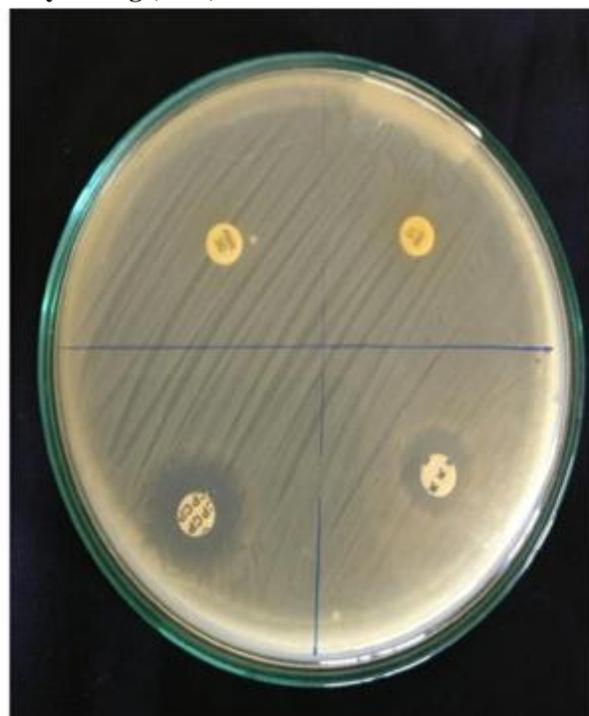
Figure 2: Antibiotic Susceptibility testing (AST)AST of *Pantoea agglomerans*AST of *Klebsiella oxytoca*

Table 2: Antibiotic susceptibility testing (AST)

Total number of isolates subjected to AST = 20

Gram negative Isolates	Antibiotic Disc Used											
	GEN (10 mcg)			TE (30 mcg)			CAZ (30 mcg)			IPM (10 mcg)		
	S	I	R	S	I	R	S	I	R	S	I	R
<i>Klebsiella pneumoniae</i> n = 6	1	4	1	0	0	6	0	0	6	0	0	6
<i>Pantoea agglomerans</i> n = 8	1	6	1	0	2	6	0	0	8	0	0	8
<i>Serratia marcescens</i> n = 6	0	1	5	1	4	1	0	0	6	0	0	6

Abbreviation:

n = Number of samples subjected to AST

S = Sensitive, R = Resistant, I = Intermediate

DISCUSSION

The risk of serious food poisoning outbreaks linked to street foods remains a threat in many parts of the world. A lack of knowledge among street food vendors about the causes of food-borne disease is a major risk factor. Considering all these factors, study was conducted in Ulhasnagar region of Thane district in order to make consumers aware of hygiene and health hazards associated with consumption of contaminated street food products. Similar studies were conducted by Mirriam E. Nyenje et al. in the states of South Africa⁴.

Different samples from various outlets were selected for determination of microbial contamination. Different samples were processed differently. For solid samples like (ragda of pani-puri, samosa, vada, etc.), they were weighed 5g each and suspended in 5 ml of sterile physiological saline¹. This mixture was subjected to centrifugation at 2000 rpm for 5 minutes¹. For liquid samples like fruit juices, milkshakes, chutneys, etc. 5ml of each sample was taken in a centrifuge tube and centrifuged at 2000 rpm for 5 minutes. After centrifugation, the supernatant was discarded and sediment was used for isolation on nutrient agar and selective medium. Selective media like MacConkey's agar, Salmonella Shigella agar, Mannitol Salt agar and Nutrient

agar were used for isolation. Shejwan chutney samples from college canteen source were found to be contaminated with *Salmonella* and *Shigella* spp. The fruit juices were contaminated with *Pantoea agglomerans*, *Serratia marcescens* and *Klebsiella oxytoca*¹⁷. *Escherichia coli* contamination was not found in any of the tested food products. In the study conducted by Mirriam E. Nyenje, Collins E. Odjajare on ready-to-eat foods from roadside café in South Africa found out that vegetables, rice, potatoes, beef and chicken products were heavily contaminated with pathogenic organisms. Organisms isolated included *Listeria* spp., *Enterobacter* spp., *Aeromonas* spp., *Proteus mirabilis*, *Klebsiella* spp., *Staphylococcus aureus*, *Pseudomonas kuteola*. Interestingly, even they did not isolate any *Salmonella* strains and *Escherichia coli* in any of the tested food products. This indicated that the fecal contamination was absent⁴.

The colony appearance of all the samples streaked on MacConkey's agar, Salmonella Shigella agar and Mannitol Salt agar along with microscopic analysis of gram staining of the colonies were observed. Colony appearance on MacConkey's agar were mucoid, pink (lactose fermenting) and colourless (non-lactose fermenting). Some of biochemical tests like Indole test, Methyl red test, Voges Proskauer's test, Simmon Citrate

test, Motility tests and Oxidase test were performed. These tests were chosen based on the literature available as well as the chemicals available. The microscopic analysis revealed that the organisms were gram negative in nature and in cocco-bacilli shape. Organisms isolated were of mixed type in nature, some were able to ferment lactose while some were not able to ferment lactose. Depending upon the biochemical test results, the identified isolates were *Enterobacter aerogens* and *Enterobacter cloacae* in pine apple juice, sweet chutney and sweet lassi. *Klebsiella pneumoniae* and *Klebsiella oxytoca* in chocolate milkshake, sweet chutney, shejwan chutney, coconut chutney and in sugarcane juice. *Pantoea agglomerans* was the mostly commonly isolated organism in most of the tested food samples. *Citrobacter koseri* was found in spicy chutney of panipuri. Similar studies were conducted by Madhuchhanda Das, Chandi C. Rath, U. B. Mohapatra in Orissa. They isolated *Enterococcus spp.*, *Bacillus spp.*, *Enterobacter spp.*, *Micrococcus tetragens*, *Salmonella paratyphi*, *Shigelladysenteriae* and *Vibrio spp.* in khattapani and smashed potato masala (ragda) of panipuri taken from various locations in Baripada city².

The identified isolates have varying degree of pathogenicity. To name few, *Pantoea agglomerans* serves as a plant pathogen. Consumption of plant infected with this organism can diseased state condition. In the study of Chandi C. Rath it was found out that the organism *Pantoea agglomerans* is an opportunistic pathogen in immunocompromised patients. Infections caused are contaminated intravenous fluid, blood stream infection, septic arthritis, periostitis, endocarditis and osteomyelitis in humans. *Klebsiella spp.* is also an opportunistic pathogen. The most common condition caused by infection of *Klebsiella spp.* is bronchopneumoniae, empyema, lung abscess, cavitation. It also causes urinary tract infection, thrombophlebitis, diarrhoea, osteomyelitis, meningitis and bacteremia and septicemia. *Enterobacter cloacae* is a member

of the normal gut flora of many humans and does not cause any disease. However some strains have been found to be associated with urinary tract infection and respiratory tract infections. *Hafnia alvei* is also an opportunistic pathogen of facultative anaerobic in nature. It is widely responsible for food spoilage and causes nosocomial infection, septicemia, respiratory tract infection and urinary tract infections. *Citrobacter koseri* is known to cause wide range of nosocomial infections of respiratory tract, urinary tract and the blood. *Citrobacter koseri* is a normal flora of human and animal digestive tract. *Citrobacter koseri* has a unique ability to penetrate, survive and replicate into vascular endothelial cells and macrophages. It also survives in phagolysosomal fusions and replicates within the macrophages which mainly contributes to the establishment of chronic abscesses.

Antibiotic susceptibility testing (AST) of the most commonly isolated organisms from the tested food samples was performed. The basic aim to carry out AST was to determine the sensitivity of the isolated organisms from ready to eat street food against most commonly prescribed antibiotics.¹⁹ Antibiotics like Tetracycline (TE), Ceftazidime (CAZ), Gentamycin (GEN), Imipenem (IPM). Gentamycin, Imipenem antibiotics of potency 10 mcg were used and Ceftazidime, Tetracycline of potency 30 mcg were used. Muller-Hinton Agar was used for antibiotic susceptibility testing for the simple fact that it avoids any fungal contamination. Mainly three commonly found isolates were subjected to AST, *Klebsiella pneumoniae*, *Pantoea agglomerans* and *Serratia marcescens*¹⁸. Out of the tested isolates, *Klebsiella pneumoniae* and *Pantoea agglomerans* were found to be resistant to Tetracycline, Ceftazidime, Imipenem and intermediately sensitive to antibiotic Gentamycin. *Serratia marcescens* was found to be resistant to Gentamycin, Ceftazidime, Imipenem and intermediate sensitivity towards antibiotic Tetracycline.¹⁸ A study conducted by Kabiru Olusegun Akinyemi et al stated that

Street vended foods were potential reservoirs for spread of emerging strains of drug resistant bacteria in Nigeria. They tested seventy six bacterial isolates made up of six genera. *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter spp.*, *Serratia marcescens* and *Proteus spp.*³. They found out that most of the isolates were moderately sensitive to Cotrimoxazole, Nalidixic acid, Amoxicillin and Nitrofurantoin. Also over 60% of pathogens isolated were inhibited by ceftazidime, cefpodoxime and levofloxacin antibiotics³. Similar study was conducted by J. M. Miranda et al to detect the presence of antimicrobial resistance of *Escherichia coli* isolated from food stuffs from grocery stores and supermarkets in Mexico. Total 10 antibiotics were used for testing and they found out that, *Escherichia coli* strain isolated from chicken, meat had higher levels of antimicrobial resistance against all the antibiotics tested⁵.

CONCLUSION

Total 55 samples were processed which included various ready to eat street food products like, vada, samosa, uthappa, fruit juices, sweet chutney, shejwan chutney, samashed potato masala, etc. Selective medias and biochemical tests were used to detect the coliforms in the test samples. 30 samples were used for isolation and detection of coliforms. The isolated organisms were, *Serratia marcescens* (13%), *Klebsiella pneumoniae* (23%), *Klebsiella oxytoca* (10%), *Pantoea agglomerans* (37%), *Enterobacter spp.* (10%), *Citrobacter koseri* (3.5%), *Hafnia alvei* (3.5%).

Commonly isolated organisms (*Pantoea agglomerans*, *Serratia marcescens* and *Klebsiella pneumoniae*) were sub-cultured on Nutrient agar slant and further used for AST. Most of the isolates were resistant to antibiotics like Ceftazidime, Tetracycline and Imipenem. Continuous consumption of food contaminated with such organisms may lead to spread of emerging drug resistant strains.

The tested food samples did not show presence of *Escherichia coli*, *Salmonella spp.*

which indicated that the fecal contamination is absent. Freshly prepared fruit juice samples were found to have less coliform count. Hence it is recommended to cut down the storage time for ready to eat food products.

Continuous analysis of street vended food samples specially unprocessed samples should be done so as to check overall load of microorganisms, presence of pathogenic organisms indicating fecal or any other contamination as well as emergence of drug resistant microorganisms. This data will help in educating street vendors to use good quality water, cut down storage time & keep the samples away from exposure to dust and external environment so that it remains safe & healthy for consumption.

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