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BACTERIAL PROFILE OF STREET VENDED PANIPURI FROM DIFFERENT ZONES OF JABALPUR CITY OF MP, INDIA

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ABSTRACT

India is a country where each city boasts of its own unique street food and a large percentage of population relishes on these delicacies. The most popular street food in India is panipuri. The present study undertaken to assess the bacterial profile of masala pani and matar sold with panipuri in Jabalpur city of Madhya Pradesh, India. Total twelve samples from different zones of Jabalpur were aseptically collected and analysed within one hour of procurement. Isolation, enumeration and identification of the bacteria were carried out following the standard procedure. Result shows that the total viable count of bacteria varied between 58.6-121.3×$^{10-5}$CFU and 48-119.3×$^{10-5}$CFU in all samples of masala pani and matar respectively, this showed that almost all panipuri samples have high bacterial load of pathogenic bacteria such as *Enterobactor facellius, Salmonella sp., Pseudeomonasflourosence, Protease sp., Streptococcus sp., E. coli, Enterobactoraerogenous* and *Klebsella pneumonia*. The mean scores of total viable count (CFU) of masala pani from four zones of Jabalpur do not differ significantly ($p \leq 0.05$) and mean scores of total viable count (CFU) of matar from four zones of Jabalpur differ significantly ($p \geq 0.05$).thus, bacterial profile demonstrates that the panipuri sold in Jabalpur city constitute an important potential hazard to human health and provision of health education to the vendors improve quality of panipuri.

Keywords: Panipuri, street food, bacteria, matar.

INTRODUCTION

Street food are ready to eat foods and beverages, prepared and sold by vendors particularly in street and similar places. These are extremely popular in worldwide and provide variety of food and readily accessibleat a cheaper price. Around worldwide 2.5 billion people consume street food everyday (FAO 2007).

India is famous for their unique street food and millions of people consume a wide variety of

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ready to eat street foods and beverage. In developing countries like India Street food contributes to 40 % of daily diet of urban population (consumer international, 2011; Pma1). People who depends on these types of food are often more interested in its convenience rather than its safety, quality and hygiene. All types of foods are sold by street food vendors, they also provide variety and choice to customers.

Consumption of this type of foods potentially increases the risk of food borne diseases caused by various pathogens. Usually vendors sold these foods by wheels barrows, trays mats, tables and make shift stalls consequently, they increasing the risk of food contamination(Ray and Mishra 2014).Contamination also from raw material and equipments, additional processing conditions, improper handling and prevalence of unhygienic conditions contribute significantly to the entry of food borne pathogens. The potential for the contamination of street food with pathogenic microorganism has been well documented and several disease outbreaks have been traced to consumption of contaminated street foods. (Abdussalamand Kaferstein, 1993). Microbial contamination of street food is an indicator of poor sanitary practices in the preparation and storage of the food. (Saxena and Agrawal, 2013).

In India street food hawkers are commonly unaware of food regulations and untrained in food related matters as well as disease outbreaks as they are from very rural background and most of them are illiterate. Today, street food has become one of the major concern of public health and a focus for governments and scientists to raise public awareness. (Sharma and Mazumdar, 2014). Among all type of street foods, panipuri or golguppa is traditional and very popular in all cities of India, and are consumed by huge population and frequently associated with food borne illness due to their improper handling and serving

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practices. Therefore, taking these factors into account this study was undertaken to assessed the bacteriological quality of panipuri, which lavishly consumed at street sides in Jabalpur city, MP, India.

MATARIAL AND METHOD:

Material

Nutrient Agar, various selective and differentiated media and IMVic Test Kit were procured from Hi-Media, Mumbai and prepared as per Manufacture's instruction.

Sample site and Sample collection

Microbiological investigation of panipuri in Jabalpur city were performed during March- June 2016. The study was conducted in different zones of Jabalpur city, MP. Each sample of panipuri was fragmented into two different segments (the liquid masalapani and solid matar masala) and were collected in sterile plastic containers, which were sealed and transported to the laboratory and processed within 1 hour of collection.

Microbiological Analysis

For the microbiological analysis of food samples, dilution was made according to the method given by Agrawal & Hasija, 1986. After serial dilution pour plate technique was applied on nutrient agar. After solidifying, Petri plates were incubated at 37°C for 24 hours in inverted position in B.O.D incubator. After completing the incubation period, count the colonies appeared on the surface of NA media in the Petri plates. This number is designated as colony forming unit (CFU) and it can be calculated by using the following formula (Verma and Verma, 2016).

$$CFU/g = \frac{\text{Number of colonies}}{\text{Weight of sample}} \times \text{Dilution factor}$$

The isolated colonies of organism were transferred to nutrient agar slant for maintenance and further identification.

Identification and isolation of Bacteria

Gram staining, growth on selective & differential media and Biochemical tests for various bacterial isolates were done for identification of bacteria. After identification, pure culture stored

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in the culture collection centre in laboratory for further use and maintenance.

Statistical analysis

Statistical analysis was done using statistical package for the social sciences (SPSS) 16.0 version. All the values were expressed as Mean, SD and One-way ANOVA. Statistical significance level was considered to be present when the two-tailed probability was less than 0.05.

RESULT AND DISCUSSION

A total of twelve panipuri samples from different zone were analysed for presence of bacterial pathogens by pour plate technique at different dilutions. The total viable count (CFU/ml) obtained at different dilution is compiled in table 1. Figure no. 1. Mean and SD value oftotal viablecount of 10^{-5} dilution in masala paniand matar. Result shows that the total viable count of bacteria varied between 58.6-121.3×$^{10-5}$CFU and 48-119.3×$^{10-5}$CFU in all samples of masala pani and matar respectively, this indicates that almost all panipuri samples have high bacterial load. Table no. 3 & 4 indicates the Zone- wise comparisons of mean scores of total viable count (CFU) in masala pani and matar respectively. The result shows that the mean scores of total viable count (CFU)of masala pani from four zones of Jabalpur do not differ significantly($p \leq 0.05$)and mean scores of total viable count (CFU) of matar from four zones of Jabalpur differ significantly ($p \geq 0.05$).This can be linked to contamination factors such as equipment and utensils with inadequate hygienic condition, utensils uncovered, garbage bin left open and in unsuitable places, irregularity of hand washing, inappropriate processing, incomplete heating, use of contaminated water during preparation and washing or secondary contamination via contact with contaminated equipment's such as chopping boards, knives and serving wares (Derbew*et al.*,2013; Mahale et al., 2008; Wai Q et al.,2006). This might also implicate the processing

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and rinsing water as possible sources of contamination of panipuri sold by street vendors (Das *et al.,*2010).

The majority of samples were found to contain the presence of different species of pathogenic microorganism indicating poor bacteriological quality of the food samples. Isolation was done by streaking selected colonies on different selective media plates Eosin Methylene Blue Agar (EMB), MacConkey Agar, Cetrimide Agar, Salmonella Shigella Agar and Blood Agar for obtaining pure culture of the isolates result obtained is represented in figure no. 2. table no. 5 shows the gram staining, morphological structure of identified bacterial isolates. Table no 6 shows the result of Biochemical test of pure culture isolates. Based on the growth on selective and differential media and biochemical tests, various bacterial isolates were identified as *Enterobactor facellius, Salmonella sp., Pseudeomonasflourosence, Protease sp., Streptococcus sp., E. coli, Enterobactor aerogenous* and *Klebsella pneumonia.* Saxena *et al.* (2016) & Gulati and Chakraborty (2017) are also found similar results in their studies.The bacterial contamination in panipuri is because of the conditions under which it is prepared and vended. In most of the cases running water is not available at vending sites and thus hand and dish washing are usually done in buckets and sometimes without soaps. (Das et al., 2012).

The occurrence of *e. coli, Enterobacter sp., klebsiella pneumonia and p. aeruginosa*may be due to poor personal hygiene of vendors, unhygienic handling of foods, poorly cleaned dishes and use of raw vegetables like onion etc. contaminated water supplies, vehicular transmission and sewage. (Tamberkar et al., 2011). The detection of respiratory pathogens such as Klebisella sp. in panipuri attributed to the bacterial aerosols generated due to sneezing and coughing in public places. (Das et al.,2010). Das et al (2010) showed that street food such as panipuri, bhelpuri and chaat in

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Bangalore city, were contaminated with high loads of pathogens that is *Streptococcus faecalis, E. Coli, Staphylococcus aureus, Bacillus sp. klebsiella sp. and pseudomonas sp.*, which similar with our findings.Hence, it is concluded that panipuri was contaminated with as *Enterobactorfacellius, Salmonella sp., Pseudeomonasflourosence, Protease sp., Streptococcus sp., E. coli, Enterobactoraerogenous* and *Klebsella pneumonia* which cause various food borne diseases.

CONCLUSION

Panipuri is very popular street food of India which is consumed by large amount of population. For street food contamination vendors personal hygiene is also responsible. As vendors touch the floor, wash the utensil most of the time without using soap, handling of dish cloths and after all they touch food without glows for preparing and serving food without washing their hands, this may lead to cross contamination of pathogenic bacteria. The present study shows high level of contamination in one of the most popular Indian street food, panipuri in different zones of Jabalpur city. The study throws a light on the potential risk of food poisoning, to overcome this alarming situation, vendors should be taught to good manufacturing practice and good hygiene practice to reduce street foods contamination. Regular monitoring of the conditions of street vended is necessary. The local government and the ministry should consider establishment of adequate facility and utility service as well as provision of necessary information, education and training programmes for vendors and consumers.

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Table 1: Zone-wise Bacterial Count (CFU) of Panipuri (pani & Matar) in Different Dilutions factor

	Sample Code / Dilution Factors	Zone A			Zone B			Zone C			Zone D		
		10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
panipuri–masala pani	P1	110	79	61	61	50	48	97	76	43	132	101	77
	P2	100	97	76	119	89	65	119	84	81	96	74	49
	P3	127	112	95	134	109	87	124	99	74	124	99	71
panipuri-Matar	M1	107	100	98	82	42	16	102	90	58	114	97	56
	M2	121	109	90	99	76	45	120	72	68	128	103	89
	M3	109	89	73	78	62	39	87	69	57	120	103	93

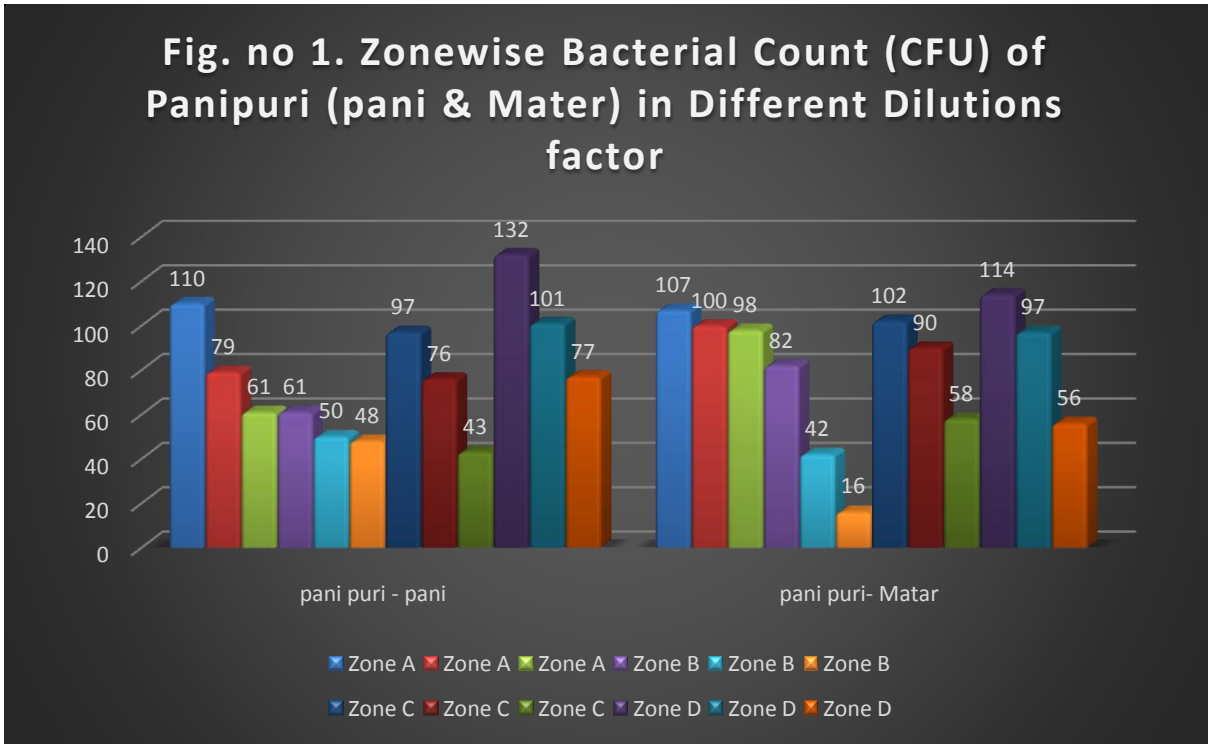

Table 2: Mean and SD of bacteria Count (CFU×10^{-5}) in Panipuri (pani & Matar)

		zone a	zone b	zone c	zone d
panipuri- pani	s1	97.3±2.5	58.6±2.5	73±3	99±2
	s2	121.3±5.13	91±2	82.6±1.5	75±1
	s3	80.3±1.5	110.3±2.5	96±2.6	99±2
panipuri- matar	s1	101.6±4.7	48±1.5	70±2	95.6±1.5
	s2	119.3±.1.52	77.6±2	88±2	100.3±2.5
	s3	108.3±3	63±4.5	64.3±5	99.6±3

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Table 3: Zone wise comparison of bacterial count (CFU) of panipuri - masala pani

CFU- masala Pani	df	Sum of square	MSS	F-value	significance
Levels of zone	3	427.5	142.528	.398	Non-Significant at 0.05level
Error	8	2862.2	357.787		
Total	11				

Table 4: Zone wise comparison of bacterial count (CFU) of panipuri - matar

CFU- Matar	Df	Sum of square	MSS	F-value	significance
Levels of zone	3	4178.5	1392.849	12.278	Significant at 0.5 level
Error	8	907.5	113.444		
Total	11				

Table 5: No. of isolates, Gram Staining, Morphological structure and identified Bacteria from panipuri (Pani and Matar)

Food sample	No. of isolates	Gram staining	Morphological structure	Identified bacteria
Panipuri – masala pani	6	-	Rod chain	*Enterobactorfacellius*
		-	Rod	*Salmonella sp.*
		-	Rod chain	*Pseudeomonasflourosence*
		-	Rod chain	*Protease sp.*
		+	Coccus	*Streptococcus sp.*
		-	Rod	*E. coli*
Matar	4	-	Rod	*Enterobactoraerogenous*
		-	Rod	*E. coli*
		-	Rod	*Klebsella pneumonia*
		-	Rod	*Salmonella sp.*

Table 6: Biochemical test results of identified bacteria

	gram stain	ind	Mr	vp	Cat	glu	suc	cit	mot	amy	Ure	cas	Oxi
E coli	-	+	+	-	+	+	+	-	+	-	-	-	-
Salmonella sp.	-	-	+	-	+	+	-	-	+	-	-	-	-
Klebsiellasp	-	-	-	-	+	+	+	+	-	+	+	-	-
Pseudeomonasflourosence	-	-	-	-	+	-	-	+	+	+	-	+	-
Proteus sp.	-	-	+	-	+	+	+	V	+	+	+	+	-
Staphylococcus sp.	+	-	+	V	V	+		-	-	-	+	+	-
Enterobacter aerogenes	-	-	+	-	+	+	+	+	+	-	-	-	-
Enterobacter faecalis	-	-	-	+	+	+	+	+	+	-	V	-	-

Note: Ind-indole, Mr-methyl red, vp- Voges-Proskauer, Cat-Catalase, glu-glucose,
suc- sucrose, cit- citrate, mot-motility,amy-amylase, ure-urease, cas-casinase, Oxi-Oxidase
v- 11-89% positive

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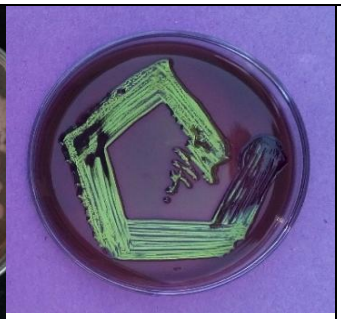
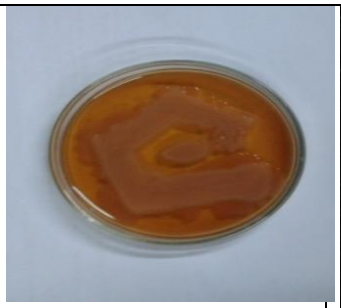
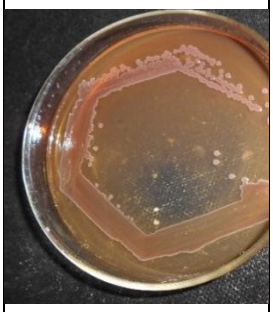
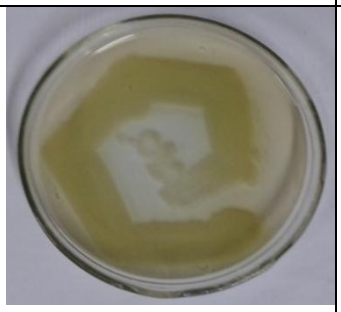
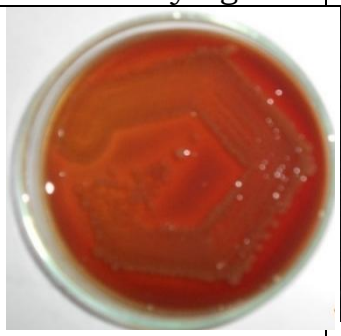
Figure No 2 : Master Plates of Bacterial Isolates

			
*Klebsiella*sp. in Mac. Agar	*Ecoli* in EMB agar	*EnterobactorAerogenoues*in MacConkey Agar	*Protease* Sp. in Blood Agar
			
Salmonella sp. in SS Agar	*Pseudeomonasflourosence*in Cetrimide Agar	*Streptococcus sp.* in Blood Agar	*EnterobactorFaceliius*in Mac. Agar

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