

Prevalence and antibiotic susceptibility pattern of *Escherichia coli* isolated from Panipuri water samples in Akola city

Barate D.L. and Samal S.N..

Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola-444001 (M.S.)

Abstract

Panipuri is one of the most popular street vended foods which is found everywhere in India. The present study attempts to assess the prevalence and antibiotic susceptibility pattern of *Escherichia coli* from the Panipuri water samples in the city of Akola, Maharashtra to understand the associated risks of consuming Panipuri from the street vendors. Fifty Panipuri samples were collected from different locations of the Akola city and analyzed for the prevalence and antibiotic susceptibility. The prevalence of *Escherichia coli* isolated from Panipuri samples was found to be 88% as 44 out of 50 samples being positive for *Escherichia coli*. The number of samples showing MPN/100ml count below 10 were 27.27%. It was found that 59.09% of Panipuri water samples showed MPN/100ml count ranging from 11-100 and 13.63% samples showing MPN/100ml count above 100. The isolates were tested against 7 different antibiotics. It was found out that the isolates showed 100% resistance against Ampicillin and Amoxycylav; high sensitivity was showed by isolated against Ciprofloxacin, Gentamicin and Chloramphenicol as 86%, 70%, and 70% respectively whereas Erythromycin and Tetracycline showed intermediate resistance with values of 23% and 9% respectively. The *Escherichia coli* found in these Panipuri water samples had many multi drug resistant strains. This study shows the potential risks of consuming such contaminated food which can cause severe illness.

Keywords- Panipuri, *Escherichia coli*, antibiotic susceptibility

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 accessible at a cheaper price. Around worldwide 2.5 billion people consume street food every day. In present times, people in urban parts of India are dependent on ready to eat street food which is easily available and economical as well (Madhuchhanda *et al.*, 2012). Consumption of these type of foods potentially increases the risk of food borne diseases caused by various pathogens. Usually vendors sell these foods by wheels barrows, trays mats, tables and make shift stalls consequently, they increase the risk of food contamination (Ray and Mishra 2014). The microbiological status of the food has been reported to be dependent on several factors like quality of raw material, handling and processing of food, presence of microorganisms that survive the preservation and storage treatment and post process contamination. Besides direct health consequences, these food borne illnesses can reduce the productivity and economic output, and also impose substantial stress on health care system. Panipuri, which is known as Gulgappa and by some other names, comprises three different components: 1) gulgappa/patasha/puri/papri, 2) filling or masala, 3) spicy water or pani. Wheat flour is used to make Puri. In Masala boiled or mashed potatoes mixed with spices are used. The Pain is sour water to which spices like salt, pepper mango powder, jaljeera etc. are added. In every Puri, Masala are added and then spicy water is filler in this Puri and served to the consumers in plate. Although Panipuri is very popular, easily available and cheap, it is frequently associated with various food borne diseases in India and elsewhere (FAO 1988, Estrada-Garcia *et al.*, 2004, Chumber *et al.*, 2007, Ghosh *et al.*, 2007). Unhygienic Panipuri is harbored by potentially life-threatening bacteria like *Salmonella typhi* and *Escherichia coli* (Garode and Waghode, 2012). Vendors can be the carriers of pathogens like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* (Tambekar *et al.*, 2011).

Escherichia coli is a consistent inhabitant of the human intestinal tract and it is the predominant facultative organism in the human gastrointestinal tract. It is considered as a reliable indicator organism of fecal pollution, generally in insanitary conditions of water, food, milk and other dairy products. But some strains, when they enter fecal-oral route may cause illness sue to toxins they secrete. Due to the complex biochemical composition and water

activity, street-vended foods like Panipuri acts as an excellent culture medium for the growth and multiplication of *Escherichia coli*. Contamination of Panipuri from clinical isolated of *Escherichia coli* can be life threatening due to their resistance to antibiotics.

Material and Methods:

1). Collection of samples :

Study site and sample collection: The bacteriological assessment of Panipuri in Akola city were performed during January – March 2024. The study was conducted across the major streets, markets, vended stalls, Samples were collected from various places/sites in different areas and localities. A total of 50 panipuri water samples were collected from various selected vending sites.

Method of sample collection: The samples were collected in sterilized zip lock polybags and kept in an ice bucket and transported aseptically to the laboratory within 1 hour. In the laboratory the samples were processed immediately following standard procedure.

2) Isolation and Identification of *Escherichia coli* from sample:

The isolation in the case of panipuri water sample is done via the collecting the sample with sterile inoculating loop and placing it onto the sterile culture media using lawn culture method. The loopful of the pani sample was directly inoculated onto culture media without homogenization. In the isolation of bacteria; 100 ml of Nutrient Agar, 100 ml of MacConkey Agar, 100ml of Eosin Methylene Blue Agar were prepared and were autoclaved at 15lbs and 121⁰ C for 15 minutes. After autoclaving the media, these media are allowed to cool down, then they are poured into sterile petri plates. Each media is poured into 2 plates. These plates are then allowed to be solidified plates and after the lawn culturing of the samples the plates were kept in incubator for 24 hours in at 37⁰C. The colonies are observed after 24 hours and are re-streaked to obtain pure culture colonies. The isolates were then identified by morphologica, cultural and biochemical characteristics as per the Bergey's Manual of Determinative Bacteriology.

3) Most probable number (MPN) count for coliforms:

Most Probable Number (MPN) count was done by inoculating each panipuri sample in set of test tubes containing single and double strength lactose broth. The no. of tubes showing positive acid gas production were then matched with MacCardy's table.

4) Determination of the antibiotic susceptibility pattern of *Escherichia coli* isolates:

The antibiotic susceptibility pattern is confirmed via performing the antibiotic susceptibility test which is performed by Kirby-Bauer disc diffusion method recommended by Clinical and Laboratory Standards Institute using Mueller-Hinton agar (CLSI, 2015). Antibiotics such as Ciprofloxacin (5µg), Gentamicin (10µg), Ampicillin (10µg), Chloramphenicol (30µg), Erythromycin (15µg), Amoxycylav (30µg), and Tetracycline (30µg) were tested against *Escherichia coli*. Resistance to three or more than three classes of antibiotics tested was considered as MDR.

Results and Discussion:

A total of 50 Panipuri water samples were collected from different Panipuri hawkers and vendors from different areas of Akola city (Table 1). Out of all the samples collected *Escherichia coli* was isolated from 44 samples whereas the remaining 6 samples were negative as no isolates of *Escherichia coli* were found. The isolates were identified as per the Bergey's Manual of Determinative bacteriology (Table 2). The prevalence of *Escherichia coli* in the samples was found to be high which was 88% (Fig. 1). The other studies also highlighted prevalence of *Escherichia coli* as

Tambekar *et al.*, (2011) recorded around 93% and Rani *et al.*, (2018) recorded the *Escherichia coli* contamination was 30%.

The samples were subjected to determination of total coliform count by Most Probable Number (MPN) technique. The total number of positive tubes of Lactose broth (10ml, 1ml, 0.1ml) were then matched with the McCardy's Table and the value of MPN/100ml was calculated. Table 3 shows the MPN/100ml count for various Panipuri water samples for coliform. It was found that the number of samples showing MPN/100ml count below 10 were 12 (27.27%), 26 (59.09%) of Panipuri water samples showed MPN/100ml count ranging from 11-100 and the number of samples showing MPN/100ml count above 100 were 6 (13.63%). High coliform count in the water samples were suggested that the water samples were unsafe for consumption as per the Bureau of Indian Standards. It stated that a coliform count zero was regarded as safe; less than 10 is considered as low risk; 11-100 is considered as medium risk whereas above 100 is considered as high risk and unsafe for consumption. Similarly Kulshreshtha (2023) found out that majority of the MPN count of coliform bacteria in golgappa water was found in the range of 110 to 160 which was higher as per the described limit.

The isolates were also checked for antibiotic susceptibility pattern by Kirby-Bauer disc diffusion method (Fig. 2).. The isolates were tested against 7 different antibiotics which were Ciprofloxacin, Gentamicin, Ampicillin, Chloramphenicol, Erythromycin, Tetracycline and Amoxycylav. It was found out that the isolates showed 100% resistance against Ampicillin and Amoxycylav; high sensitivity was showed by isolated against Ciprofloxacin, Gentamicin and Chloramphenicol as 86%, 70%, and 70% respectively whereas Erythromycin and Tetracycline showed intermediate resistance with values of 23% and 9% respectively. It was found that most of the isolates showed multiple drug resistance. Ghimire *et al* (2021) also reported that 52.9% of *Escherichia coli* were resistant to more than 3 different classes of antibiotics and hence were considered to be multidrug resistant strains.

Table 1: Collection of Panipuri water samples from various sites of Akola city.

Serial Number	Sample Code	Area of Collection	Presence of <i>Escherichia coli</i>
1	PW01	Chhoti Umari	Negative
2	PW02	Chhoti Umari	Positive
3	PW03	Chhoti Umari	Negative
4	PW04	Chhoti Umari	Positive
5	PW05	Gandhi Road	Positive
6	PW06	Radhakishan Plots	Positive
7	PW07	Radhakishan Plots	Positive
8	PW08	Radhakishan Plots	Positive
9	PW09	Radhakishan Plots	Positive
10	PW10	Radhakishan Plots	Positive
11	PW11	Tapadia Nagar	Negative
12	PW12	Tapadia Nagar	Negative

13	PW13	Tapadia Nagar	Positive
14	PW14	Tapadia Nagar	Positive
15	PW15	Ramdaspeth	Positive
16	PW16	Durga Chowk	Positive
17	PW17	Shivaji College	Positive
18	PW18	Mothi Umari	Positive
19	PW19	Mothi Umari	Positive
20	PW20	Mothi Umari	Positive
21	PW21	Kedia Plot	Positive
22	PW22	Bara Jyotirling Mandir	Positive
23	PW23	Shivani	Positive
24	PW24	Hariharpath	Positive
25	PW25	Hariharpath	Positive
26	PW26	Hariharpath	Positive
27	PW27	Barshitakli Road	Positive
28	PW28	Keshav Nagar	Negative
29	PW29	Sindhi Camp	Positive
30	PW30	Sindhi Camp	Positive
31	PW31	Sindhi Camp	Positive
32	PW32	Sindhi Camp	Positive
33	PW33	Shivaji Nagar	Negative
34	PW34	Balapur Road	Positive
35	PW35	Balapur Road	Positive
36	PW36	Gorakshan Road	Positive
37	PW37	Gorakshan Road	Positive
38	PW38	Gorakshan Road	Positive
39	PW39	Gorakshan Road	Positive
40	PW40	Gorakshan Road	Positive

41	PW41	Near Bharat Vidhyalaya	Positive
42	PW42	Ranpise Nagar	Positive
43	PW43	Jawahar Nagar Road	Positive
44	PW44	Jawahar Nagar Road	Positive
45	PW45	Jawahar Nagar Road	Positive
46	PW46	Jawahar Nagar Road	Positive
47	PW47	Jawahar Nagar Road	Positive
48	PW48	Malkapur Road	Positive
49	PW49	Kirti Nagar	Positive
50	PW50	Ratanlal Plot	Positive

Fig. 1. Prevalence of *Escherichia coli* in Panipuri water samples in Akola city.

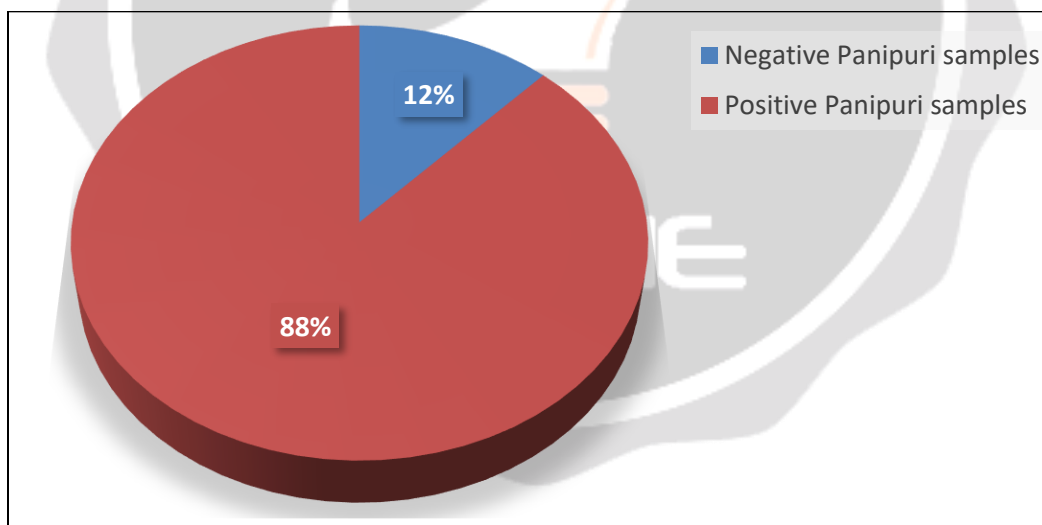


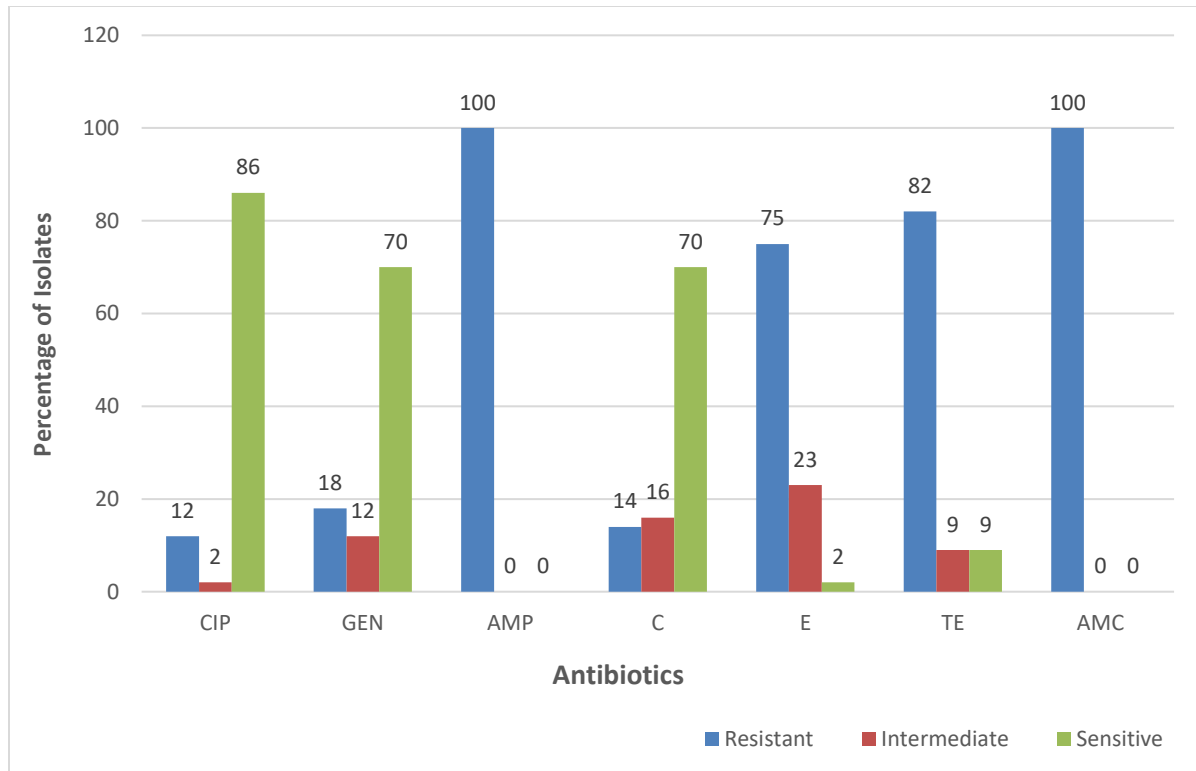
Table 2: Morphological, cultural, and biochemical characteristics of *Escherichia coli* isolated from the Panipuri water samples.

Characteristics	Isolates
Morphological character	Coccobacilli
Gram Character	
Gram Staining	Negative
Motility	Motile
Cultural Characteristics	
Size	2mm
Shape	Circular
Margin	Irregular
Color	Green Metallic Sheen
Opacity	Opaque
Elevation	Convex
Biochemical Characteristics	
Indole	+
MR	+
VP	-
Citrate	-
Sugar Fermentation (A/G)	
Lactose	+
Glucose	+
Mannitol	+
Sucrose	-
Enzyme Test	
Catalase	+
Oxidase	-
Amylase	+
Urease	-
Gelatinase	-

Table 3: Most Probable Number count of Panipuri Samples.

Serial Number	Sample Code	MPN Count			MPN/100ml
		10 ml	1 ml	0.1ml	
1	PW02	3	1	0	11
2	PW04	3	0	0	8
3	PW05	2	3	0	12
4	PW06	0	1	0	2
5	PW07	4	2	0	22
6	PW08	2	2	0	9
7	PW09	2	2	0	9
8	PW10	5	4	2	220
9	PW13	3	0	0	8
10	PW14	2	2	0	9
11	PW15	3	1	1	14
12	PW16	4	0	1	17
13	PW17	3	2	0	14
14	PW18	2	1	0	7
15	PW19	4	4	0	34
16	PW20	3	1	1	14
17	PW21	4	0	0	13
18	PW22	5	3	2	140
19	PW23	4	1	1	21
20	PW24	4	1	2	26
21	PW25	1	1	1	6
22	PW26	3	0	1	11
23	PW27	2	3	0	12
24	PW29	4	0	0	13
25	PW30	3	0	1	11
26	PW31	1	2	0	6
27	PW32	3	2	1	17
28	PW34	3	0	0	8
29	PW35	4	1	2	26
30	PW36	1	2	0	6
31	PW37	5	4	0	130
32	PW38	4	3	1	33
33	PW39	4	0	1	17
34	PW40	2	2	0	9
35	PW41	3	2	1	17
36	PW42	5	3	1	110
37	PW43	4	4	0	34
38	PW44	5	1	0	30
39	PW45	3	1	0	11
40	PW46	4	0	0	13
41	PW47	4	4	0	34
42	PW48	5	1	2	60
43	PW49	5	4	0	130
44	PW50	5	3	2	140

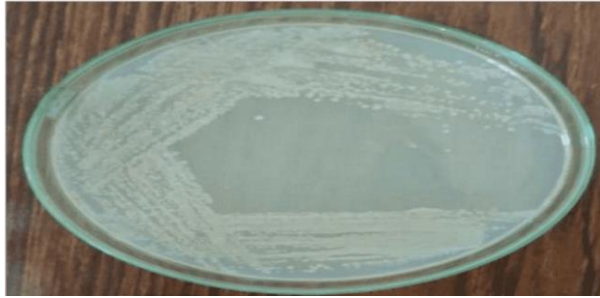
Fig. 2. Antibiotic resistance and susceptibility amongst *Escherichia coli* isolates.



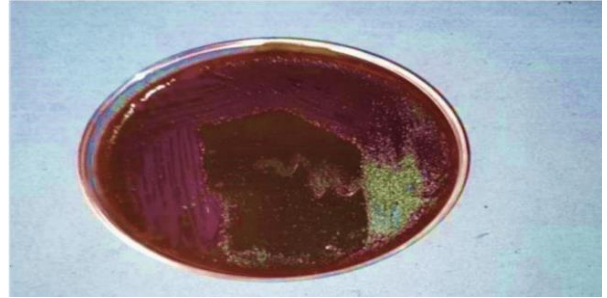
Collection of Panipuri water samples from different sites.



Isolation and Identification of *Escherichia coli*.



On Nutrient Agar



On EMB Agar



IMViC Test



Urease Test



Catalase Test



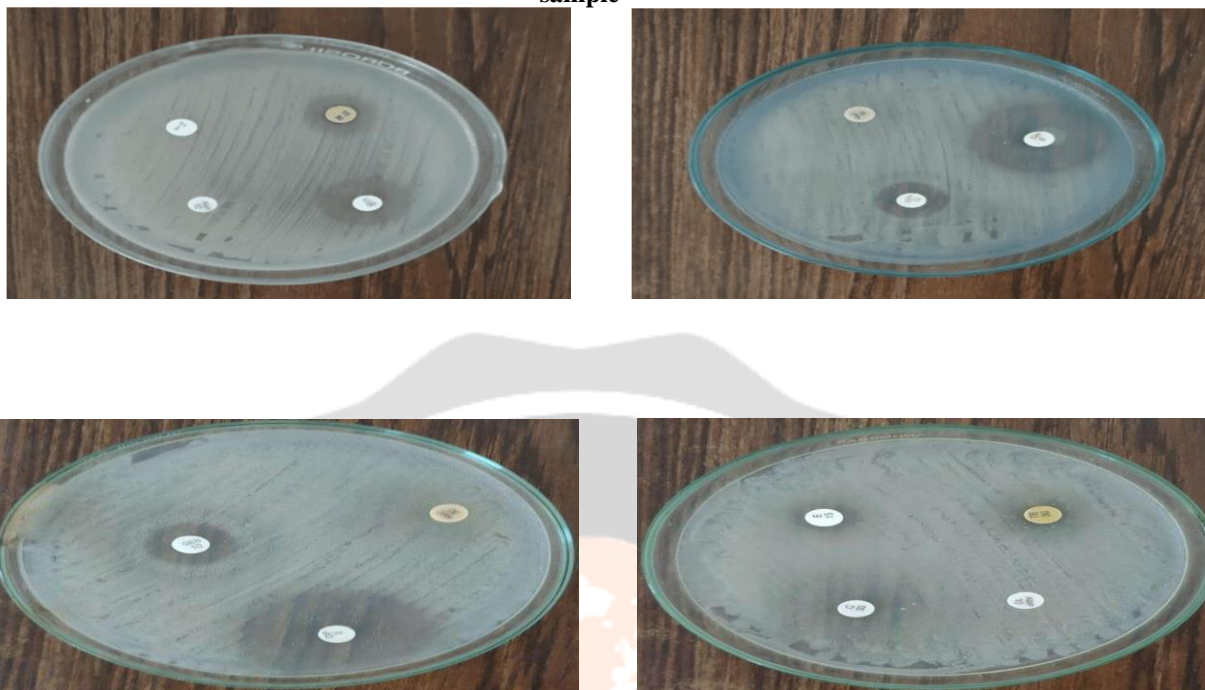
Oxidase Test



Sugar fermentation test:



Most Probable Number count of sample



Antibiotic sensitivity testing of the isolates:

Conclusion:

From the results the conclusion can be drawn that the Panipuri sold in various street side vending stalls in Akola city showed high level of contamination with high coliform counts. The strains of *Escherichia coli* found in these Panipuri water samples had many multi drug resistant strains. This study showed the potential risks of consuming such food which has contamination of this level. The contamination could be due to the unhygienic practices might be during preparing, serving or using already contaminated wate, So, regular monitoring and compliance for hygienic and sanitation practices by vendors should be mandatory and strictly conducted to maintain a good safety level for consumers.

References:

1. Chumber S, Kaushik K, and Savy S, (2007). Bacteriological analysis of street foods in Pune. *Indian J. Public Health*. 51(2), 114-116.
2. Baghel V and Singh P, (2021), Microbiological assessment of street vended panipuri water sample taken from Lucknow, India. *International Journal of basic and applied biology*; 8(1):1-4.
3. Bauer AW, Kirby WM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 45(4): 493-496.
4. Estrada-Garcia T, Lopez-Saucedo C, Zamarripa-Ayala B, Thompson MR, Gutierrez-Cogco L, Mancera-Martinez A and Escobar-Gutierrez A (2004). Prevalence of *Escherichia coli* and *Salmonella spp.* In street-vended food of open markets and general hygienic and trading practices in Mexico City. *Epidemiology and Infection* 132(6), 1181-1184.
5. Garode M. and Waghode M. (2012). Bacteriological status of street-vended foods and public health significance – A case study of Buldhana District, Maharashtra India. *ISCAJ. Biol. Sci.* 1(3) - 69-71.

6. Ghimire P, Khand S, Chaulagain, Siwakoti A, Dhakal D, Shrestha U, (2021). Microbial quality analysis of panipuri samples collected from different parts of Bhaktapur. *TUJM*; 8(1):38-45.
7. Ghosh M, Wahi S, Kumar M, Ganguli A. Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella spp.* In some raw street vended Indian foods. *Int J of Environ Heal R.* (2207); 17(2):151-156.
8. Kulshreshtha R, (2023), Evaluation of prevalence of *Escherichia coli* in street vended golgappa stalls of Bhilai, Chhattisgarh. *International Journal of Creative research thoughts*; 11(10):549-554.
9. Madhuchhanda D, Chandi R. and Mohapatra U. (2012). Bacteriology of a most popular street food and inhibitory effect of essential oils on bacterial growth. *Journal of Food Science and Technology*; 49(5), 564-571.
10. Rani B, Singh R, Sharma V, Kashikar S, Birla S, Kumar D, Singh S and Umeel, (2018), Prevalence of *Escherichia coli* isolates from different sources of water in Jabalpur city, *The Pharma Innovation Journal* ; 7(7):888-892.
11. Ray M. and Mishra P., (2014). A Preliminary study of fungi on Panipuri sold in different areas of Dhubri Town, Assam. *Trends in Biosciences*, 7(11), 991 – 993.
12. Tambekar DH, Murhekar SM, Dhanorkar DV, Gulhane PB, Dudhane MN. Quality and safety of street vended fruit juice: a case study of Amravati city, India. *J Appl Biosci.* (2009); 14(6):782-787.

