Research Article

Screening and Sequencing of Antibiotic Resistant Microorganisms from Street Food

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ABSTRACT

Food borne diseases are a great threat that involves a wide range of illnesses caused mainly through the intake of food contaminated by various agents like bacterial, viral, metals, as well as poisonous plants or chemicals. Ten samples of the edible fluid of the paani puri from ten different locations of Coimbatore during the month August to September were aseptically collected and analysed. Isolation, enumeration and characterization of the prevalent bacteria were carried out using selective and differential media, Grams staining and biochemical tests. The bacteria showing high virulence and antibiotic resistance had been taken for further studies to determine whether the antibiotic susceptibility is encoded by the genomic DNA or the plasmid DNA. Plasmid isolation procedure showed no bands in the gel stained with ethidium bromide confirmed that the antibiotic susceptibility is encoded by genomic DNA. Hence, the particular isolate was subjected for bacterial identification using 16S rRNA sequencing. The sequence was submitted to GenBank and obtained the accession number BankIt1640990 GRD KF290998. To our knowledge, the isolate is reported first time from the food sample. On this basis, we suggest the strain *Bacillus foraminis* reported earlier in the ground water is also present in the food sample that may cause food borne illness on the regular consumption of the similar food items which is being made in unhygienic conditions.

Keywords: Coli forms, Food borne diseases, Street foods, 16S rRNA gene sequencing.

INTRODUCTION

Infectious diseases spread through food or beverages are a common, distressing, and sometimes life-threatening problem. Food industry and health authorities face a threat of food poisoning that causes numerous outbreaks.[1] Food borne diseases, i.e. illnesses due to contaminated food, are one of the most widespread problems of the contemporary world. Food borne illnesses, a major public impact are infections or irritations of the gastrointestinal (GI) tract caused by food or beverages containing harmful bacteria, parasites, viruses, or chemicals.[2] They are toxic or infectious by nature and are caused by agents, which enter the body through the ingestion of contaminated food or water. These agents can be chemical like pesticide residues and toxic metals or biologicals like pathogenic microorganisms. Foods contaminated by biological agents are the major cause of food borne disease. New developments in food production and changing trends in food consumption lead to the emergence of new hazards. Each year food borne illnesses affect 6 to 80 million persons which cause 9,000 deaths, and cost an estimated 5 billion U.S. dollars.[3]

Street foods includes fast foods, junk foods, snacks, beverages, meals, salads, sliced fruits and drinks have been defined by Food and agricultural organisation as "Ready to eat foods and beverages prepared and sold by vendors especially in streets and other public places for immediate consumption".[4] The street food industry plays a very important role in meeting food requirements of commuters and urban dwellers in many cities and towns. It also provides a source of affordable nutrients to the majority of the people especially the low-income group in the developing countries.[5]

Usually simple facilities like wheel barrows, trays, mats, tables and make-shift stalls are used by vendors to sell foods. This increases the risk rate of food contamination from several factors like raw materials, equipments, additional processing conditions, improper handling and prevalence of unhygienic conditions considerably contributes to the entry of bacterial pathogens.[6] So, the food can be contaminated with microorganism may originated from the vendor's hands when they touch the food preparation areas, dish clothes and the water during dish washing and hand washing. This indicates cross contamination between dish water, food preparation surfaces, and the food itself, which perceive a major public health risk.[7-9] Street foods have become obsessive to a huge population. Mostly, the vendors are not educated regarding good hygiene practices (GHP) and the risk of causes of street foods.[10,11] Hence, screened the microbial contaminations present in the street-vended food sample, especially in paani poori since it is available in a shorter span of time in many places and it is affordable for all groups of people.

MATERIALS AND METHODS

Study sites and sample collection

Ten paani samples were collected during the period of August to September in sterile containers from ten

different street food vending shops in Coimbatore and transferred aseptically to the laboratory for further studies and analysed within an hour of procurement.

Sample analysis

1 millilitre of each of the samples was added to 9ml of 0.85% saline in a test tube and diluted serially to obtain dilutions up to 10^{-5}. Then 0.1 ml of the appropriate dilution from each tube was aseptically pipetted out and plated on different selective and differential media such as Mac-Conkey agar, Eosin Methylene Blue agar (EMB), Thiosulphate Citrate Bile Sucrose agar (TCBS), Hicrome UTI agar, Baird Parker's agar, Cetrimide agar and Salmonella Shigella agar (SS) plates using spread plate technique. The plates were kept in incubation for 48 to 72 hours at 37°C. After incubation, the microbial growth in the form of colonies was observed, manually counted and according to the colony morphology it was sub-cultured on the respective agar plates to obtain pure colonies.

Staining and biochemical identification

Ten different isolates were selected based upon the colony morphology and the colour which they produced on the agar plates; pure cultures of these colonies were maintained. Gram staining was carried out for each isolates. After staining, each of the isolates was subjected for thirteen biochemical tests to identify the genus of the micro organism based upon Bergy's manual.[12]

Virulence test of the isolates

Virulence tests such as DNAase, haemolysis, lecithinase, lipase and proteinase were performed to check the level of virulence exhibited by the food borne isolates.[12]

i. DNAse test

DNase agar supplemented with 0-0.1 per cent toluidine blue was employed as the substrate. Bacterial colonies were inoculated on the surface of the agar and allowed to incubate for 72 hours at 37°C.

ii. Haemolysis test

Five per cent defibrinated human blood was supplemented in blood agar base which was the substrate. Colonies were inoculated onto the surface of the agar and incubated at 72 hours at 37°C.

iii. Lecithinase test

Lecithinase was measured by utilising an egg yolk (50 per cent) agar base. Colonies were inoculated onto the surface of the agar and allowed to incubate for 72 hours at 37°C.

iv. Lipase test

Trypticase soy agar plates supplemented with 1 per cent tween 80 (polyoxyethylene sorbitan monooleate) served as a substrate. Colonies were inoculated onto the surface of the agar plate and allowed to incubate for 72 hours at 37°C.

v. Proteinase test

Skim milk agar was the substrate. Colonies were inoculated onto the surface of the agar and allowed to incubate for 72 hours at 37°C.

Antibiotic susceptibility of the isolates

Antibiotic susceptibility test was performed to see the susceptibility of the isolates to fourteen general antibiotics such as erythromycin, gentamicin, tetracycline, chloramphenicol, ciprofloxacin, kanamycin, ampicillin, penicillin, oxacillin, vancomycin, amikacin, piperacillin, ampicillin, amoxycillin that was prescribed for infections caused by the isolated microorganisms. Hence, these were used to determine the sensitivity towards the antibiotics.

Different cultures were inoculated in nutrient broth and incubated at 37°C for 24 hours. After which, the overnight cultures were swabbed on Muller Hinkton agar plates (MHA). The antibiotic discs were placed on the swabbed plates and incubated at 37°C for 24 to 48 hours.

Isolation of Plasmid

1. Nutrient broth medium containing appropriate antibiotic susceptible bacterial Colonies containing plasmid

2. SET buffer.

3. NaOH/SDS solution.

4. 3M Potassium Acetate (pH 4.8).

5. Buffered Phenol-Chloroform.

6. 95% and 75% v/v Ethanol.

7. De-ionised water.

8. Chloroform-isoamyl alcohol (24:1).

9. Phenol-chloroform-isoamyl alcohol (25:24:1).

10. Isoamyl alcohol.

Procedure

1. Depending on the plasmid copy number, 5 (for high-copy plasmids) - to 10 (for low-copy plasmids)-ml culture volumes were centrifuged at 10,000rpm for 5 minutes in a SorvallSS34 rotor (or its equivalent) and the supernatant was removed by aspiration.

2. Pellets were suspended by vortexing in 200ul of SET (25% sucrose, 50mM EDTA,50mM Tris-HCl [pH8.0]) and 5mg of lysozyme per ml of SET, transferred to a microcentrifuge tube, and incubated for 10 min at 37°C.

3. A 350ul volume of a fresh NaOH-sodium dodecyl sulfate solution (0.2N NaOH, 1% sodium dodecyl sulfate) was added, and the micro centrifuge tube was repeatedly inverted until the suspension cleared.

4. To the cleared suspension 350ul of a cold 3M potassium acetate solution was added. This was

rapidly inverted for *S. aureus* or vortexed for 10 seconds at medium speed for *B.subtilis.*

5. The suspension was centrifuged for 5 minutes at top speed in a micro centrifuge; and 750ul of the supernatant was transferred to a new micro centrifuge tube.
6. This supernatant fluid was extracted with 650ul of cold phenol-chloroform-isoamyl alcohol (25:24:1) by vortexing for 1 minute.
7. The mixture was centrifuged for 5 minutes, and 620ul of the aqueous phase was transferred to a new micro centrifuge tube, where it was extracted with 620ul of cold chloroform-isoamyl alcohol (24:1) by vortexing for 30 seconds. The mixture was centrifuged for 3minutes, and 550ul of the aqueous phase was transferred to a new micro centrifuge tube.
8. The plasmid DNA was precipitated by adding an equal volume of cold (-20°C) isopropanol and inverting it several times.
9. The suspension was centrifuged for 5minutes, and the isopropanol was removed by aspiration.
10. The pellet was washed with 1ml of 70% ethanol and centrifuged for 2 min. The ethanol was removed by aspiration. The pellet was dried under vacuum for 5minutes and suspended in 50ul of deionised water RNase-20 p,g/ml[7]

Purification

1. To the sample, added equal amount of chloroform: isoamylalcohol (24:1).
2. Centrifuged at 12000 rpm for 15 minutes at 4°C.
3. Transferred the top aqueous layer to fresh eppendorf.
4. Added 0.1 volume of 3M Sodium acetate and double the volume of 100% ethanol.
5. Incubated for 1 hour on ice and spin at 12000rpm for 10 minutes at 4°C.
6. Discarded the supernatant and washed the pellet with 70% ethanol, air-dry and dissolved the pellet in TE buffer.
7. Checked on agarose gel.

The isolated sample was performed with 16srRNA gene sequencing, a common tool for bacterial identification. The unknown species can be identified using BLAST (Basic Local Alignment Search Tool) to compare the query sequence against sequences present in database.

RESULTS AND DISCUSSION

Isolation of bacteria

Samples were collected from ten different shops and it was serially diluted up to 10^{-5} in saline distilled water. It was plated on different selective and differential media using spread plate technique and pure colonies were obtained (Table 1).

Table 1: Prevalence of bacteria in different locations

Media	SAMPLE. 1 Lakshmi mills					SAMPLE. 2 Fun mall					SAMPLE. 3 Hopes					SAMPLE. 4 Sitra					SAMPLE. 5 Anna statue				
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
SS	TNTC	$5.2X10^3$	$5X10^2$	2X10	1	TNTC	$5.2X10^3$	50	2	1	TNTC	$3.26X10^4$	$6X10^2$	1	NIL	$4.9X10^4$	$2X10^3$	NIL	NIL	NIL	$4.5X10^2$	43	7	2	NIL
Mac conkey	TNTC	$2.91X10^4$	$2.10X10^3$	9.2X10	49	TNTC	$1.22X10^4$	$7.6X10^2$	13	1	TNTC	$2.67X10^4$	$2.6X10^3$	21	11	$9X10^4$	$2X10^4$	$2X10^2$	NIL	NIL	$9.5X10^2$	47	7	NIL	NIL
Hichrome UTI	$2.19X10^3$	$2.91X10^4$	$1.89X10^3$	5.4X10	13	TNTC	$1.52X10^4$	$8.8X10^2$	19	NIL	TNTC	$2.23X10^4$	$1.4X10^2$	33	9	$1.3X10^3$	$9X10^2$	$7.9X10^2$	44	27	$1.48X10^3$	58	12	10	3
EMB	$1.02X10^3$	$2X10^3$	$5X10^2$	10	NIL	TNTC	$1.22X10^4$	$1.01X10^3$	40	2	TNTC	TNTC	TNTC	56	20	$1.01X10^3$	100	NIL	NIL	NIL	$5X10^2$	10	NIL	NIL	NIL
Cetrimide	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Baird parkers	$6x10^2$	$5.6x10^3$	$6x10^2$	20	2	TNTC	TNTC	$1X10^2$	42	12	TNTC	$2.41X10^4$	$9X10^2$	40	18	TNTC	TNTC	$5.8X10^2$	43	25	TNTC	TNTC	TNTC	77	49
TCBS	$2.2x10^2$	TNTC	TNTC	TNTC	TNTC	TNTC	$5.2X10^3$	50	2	1	TNTC	$6.7X10^3$	$7.6X10^2$	12	NIL	$4.9X10^4$	$2X10^3$	NIL	NIL	NIL	$1.9X10^3$	18`	NIL	NIL	NIL

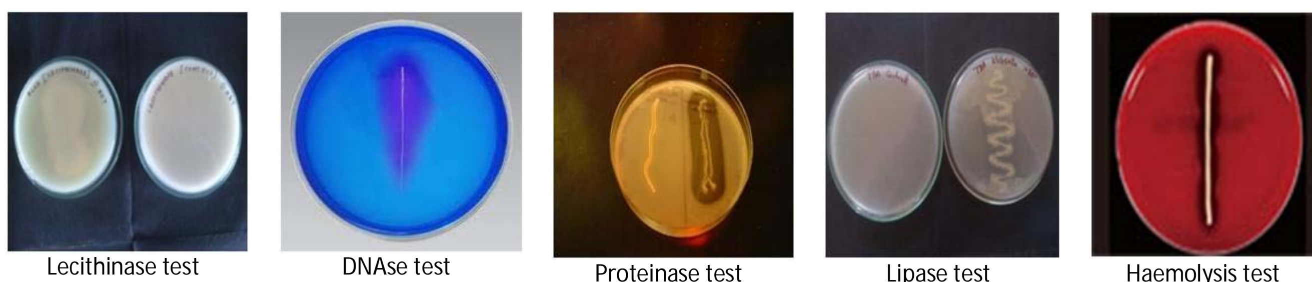
Table 1: Prevalence of bacteria in different locations (Continued.....)

Media	SAMPLE. 6 Ukkadam					SAMPLE. 7 Peelamedu					SAMPLE. 8 Krishnammal					SAMPLE. 9 Cheran managar					SAMPLE. 10 Gandhipuram				
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
SS	TNTC	$1.43X10^3$	$6.4X10^2$	6	NIL	$9.2X10^2$	$5.2X10^2$	14	5	NIL	NIL	NIL	NIL	NIL	NIL	$1.76X10^3$	$7.2X10^2$	31	5	NIL	$5.4X10^2$	$2.5X10^2$	13	6	NIL
Mac conkey	TNTC	$1.22X10^3$	$7.6X10^2$	13	1	TNTC	52	31	17	NIL	NIL	NIL	NIL	NIL	NIL	$9.5X10^2$	47	7	NIL	NIL	$9X10^2$	$5.4X10^2$	13	2	NIL
Hichrome UTI	TNTC	$1.52X10^3$	$8.8X10^2$	19	NIL	TNTC	TNTC	52	31	17	$1.82X10^3$	$1.7X10^2$	30	2	1	$1.48X10^3$	$5.8X10^2$	12	10	3	$1.3X10^3$	$9X10^2$	79	44	27
EMB	TNTC	$1.22X10^3$	$1.01X10^3$	40	2	TNTC	TNTC	$7.2X10^2$	58	23	NIL	NIL	NIL	NIL	NIL	$5.X10^2$	10	NIL	NIL	NIL	$1.01X10^3$	$4.2X10^2$	50	NIL	NIL
Cetrimide	NIL	NIL	NIL	NIL	NIL	$1.5X10^2$	40	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Baird parkers	TNTC	TNTC	$9.5X10^2$	44	28	TNTC	TNTC	$1.3X10^2$	72	54	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	77	49	TNTC	TNTC	$5.8X10^2$	43	24
TCBS	TNTC	$5.2X10^2$	$1.1X10^2$	5	1	$4.1X10^2$	20	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	$1.96X10^3$	$1.8X10^2$	NIL	NIL	NIL	$4.9X10^2$	20	NIL	NIL	NIL

Table 2: Biochemical tests of the isolates from food sample

Test	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10
Indole	-	-	-	+	-	-	-	-	+	-
Methyl red	-	-	-	+	+	+	-	+	-	-
Vogesproskeur	-	+	+	-	+	-	-	+	-	+
Citrate	+	+	+	-	+	-	+	-	-	+
Oxidase	-	-	-	-	-	-	-	+	-	-
Catalase	+	+	+	+	+	+	+	-	+	+
Gelatinase	-	-	-	-	-	-	-	+	-	-
Caesinase	-	-	-	-	-	-	-	-	-	-
Hydrogen sulphide	+	-	-	-	+	-	-	-	-	-
Carbohydrate	K	A/A	K	A/A	K/A	K/A	A/K	A/A	K/K	K
Nitrate	-	-	+	+	+	+	-	-	-	+
Urease	-	+	-	-	-	-	-	+	-	-
Starch hydrolysis	-	+	-	-	-	-	-	+	+	-

Note: - Negative, + positive, K -alkaline, A/A-acid/acid, K/A- alkaline/acid, A/K- acid/alkaline



Lecithinase test DNAse test Proteinase test Lipase test Haemolysis test

Figure 1: Virulence test of the isolates

Table 3: Rate of contamination in the samples

Organism	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
Escherichia coli.	P	P	P	P	P	P	P	P	P	P
Bacillus sp.	P	P	P	P	P	P	P	P	P	P
Staphylococcus sp.	P	P	P	P	P	P	P	P	P	P
Yersinia sp.	P	A	A	P	P	P	A	A	P	P
Klebsiella sp.	P	P	A	A	P	P	P	P	P	P
Enterobacter sp.	P	P	P	P	P	P	A	P	A	A
Salmonella sp.	A	P	A	A	A	A	P	A	A	P
Shigella sp.	A	P	P	A	A	A	P	A	A	P
Vibrio sp.	P	P	P	A	P	P	A	A	A	P
Streptococcus sp.	A	P	A	A	P	A	P	A	A	P

Note:- P-Present, A-Absent

Table 4: Antibiotic susceptibility of the food borne isolates

Antibiotics	Escherichia coli.	Yersinia sp.	Staphylococcus sp.	Bacillus sp.	Klebsiella sp.	Enterobacter sp.	Salmonella sp.	Shigella sp.	Vibrio sp.	Streptococcus sp.
Oxacillin	R	R	R	R	R	S	R	R	S	S
Amikacin	R	R	R	R	S	S	S	S	S	S
Cefazolin	S	R	R	R	R	S	S	R	S	S
Vancomycin	R	R	R	R	R	S	S	S	S	S
Chloramphenicol	R	R	R	R	R	R	R	R	S	S
Kanamycin	R	R	R	R	S	S	S	R	S	S
Gentamycin	S	R	R	R	S	S	S	S	S	S
Ampicillin	R	R	R	R	R	R	S	R	R	S
Amoxicillin	S	R	R	R	R	R	S	R	S	R
Streptomycin	R	R	R	R	S	R	S	S	S	S
Cephotaxime	S	R	R	R	R	R	R	S	R	S
Penicillin-G	R	R	R	R	R	R	R	R	S	S
Erythromycin	R	R	R	R	S	R	S	S	S	S
Pipericillin	R	R	R	R	R	R	R	R	S	S

Note:- R-Resistant, S-Sensitive

Based on the biochemical tests, Gram staining and the growth of the bacteria in different selective and differential media, the ten isolates were identified as *Escherichia coli.*, *Yersinia* sp., *Staphylococci* sp., *Klebsiella* sp., *Enterobacter* sp., *Salmonella* sp., *Shigella* sp., *Vibrio* sp., *Streptococcus* sp., and *Bacillus sp* (Table 2).

A total of ten samples were analysed. After the confirmation of the isolates through biochemical test, the rate of presence of each contaminant was analysed in the collected samples. *Escherichia coli* and *Staphylococcus* sp., and *Bacillus sp. were* found to be predominant in all the samples collected. *Enterobacter* sp., and *klebsiella* sp. were also found in most of the samples collected. Almost six samples were contaminated with the presence of *Vibrio* sp., and *Yersinia* sp. while, *Streptococcus* sp., *Shigella* sp., and *Salmonella* sp. were present in four samples (Table 3).

Virulence test of isolates

It was observed that *Bacillus* sp. was the most virulent strain isolated (Fig. 1) and antibiotic susceptibility tests showed that the *Bacillus* sp., *Staphylococcus* sp., and *Yersinia* sp., were resistant to all the antibiotics used (Table 4).

16S rRNA sequencing

Isolation of plasmid using agarose gel showed no band, which indicates the antibiotic susceptibility is encoded by genomic DNA. The particular isolate was subjected for bacterial identification using 16S rRNA sequencing. The sequence was submitted to GenBank and obtained the

accession number KF290998.1 (www.ncbi.nlm.nih.gov/nuccore/526132762) is given below. The strain *Bacillus foraminis* reported earlier in the ground water is also present in the food sample.

>gi|526132762|gb|KF290998.1| Bacillus foraminis strain KGPI 03 16S ribosomal RNA gene, partial sequence

GCATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACG
ATGCGTAGCCGACCTGAGAGGGTGATCAGCCACACTGGGAC
TGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGA
ATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGT
GAGTGAAGAAGGCTTTCGGGTCGTAAAGTTCTGTTGTAAGG
GAAGAACAAGTACCGGAGAATATGGCGGCACCTTGACGGTA
CCTGACGAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGC
GTAAAGCGCGCGCAGGCGGTCTGTTAAGTCTGATGTGAAAG
CCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGGC
TTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGT
GAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGC
GGCTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA
AACGATGACTGCTAGGTGTTGGAGGGTTTCCGCCCTTTAGTG
CTGAAGCAAACGCATTAATCACTCCCCCTGGGGAGTACGGCC
GCAAGGCTGAAACTCAAAGGATTTGACGGGGCCCCGCACAA
GCGGGGGAGCATGTGGTTTATTTCAAAGCAACGCAAAAAAC
CTTACCAGCTCTTGACGTCCTCTGACCAGCCTAGAAATAGTAC
GTTCCCCTTCGGGGGAAGGAGTGACAGGTGGTGCATGGTTG
TCATCCGCTCGTGTCGAGAGATGTTGGATTATGTCCCGCAAC
GAGCGCCCCCCTTGTTCGTAGTCGCCATCATTTGGTTGGCCTC
TCTAGGGAGACTGCCGGAGACAATACGGAGGAAGGTGGGA
ATGACCTCAAATCCTCGTGCCCCTAATGATG

TGGGCTACACACGCGCTACAGTGGA

CONCLUSION

Lack of appreciation of basic safety issues by vendors contributes to augmentation of microbial loads. The presence of microbial contaminants such as *Bacillus* sp., *Staphylococcus* sp., *Klebsiella* sp., *Escherichia coli* and *Enterobacter* sp. was high in the isolated edible fluid samples of paanipuri and upon continuous intake of the same type of food may cause food poisoning and other ill effects. The presence of *Vibrio* sp., *Pseudomonas* sp., *Salmonella* sp., *Shigella* sp., and *Yersinia* sp. in the food samples indicates that it may be contaminated with pathogenic organisms. The virulence tests showed that the *Bacillus* sp., *Staphylococcus* sp., and *Klebsiella* sp. showed more virulence when compared with other isolates. Antibiotic susceptibility tests showed that the *Bacillus* sp., *Staphylococcus* sp., and *Yersinia* sp., were resistant to all the antibiotics used.

After performing 16S rRNA gene sequencing and BLAST, the *Bacillus* sp. was confirmed to be *Bacillus foraminis*, which is a gram-positive rod shaped bacteria, aerobic and does not form spores. Previously, the organism was reported mainly from the non-saline alkaline ground water of southern Portugal and deep soils from the mines of Gujarat.[14] The same organism present in the isolates indicate the contamination of the food sample with the soil bacteria.

The presence of coliforms and *Bacillus* sp., may be due to the improper handling or the use of contaminated water for processing. It can be inferred that proper facilities and training should be given to the food vendors to provide safe food items and to control the spreading of such food borne illnesses and food poisoning.

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