Isolation and Characterization of Microbes of Different Kinds of Street Food and Determination of Antibiotic Susceptibility of the Isolates

ABSTRACT:

Aim: Improper personal hygiene can facilitate the transmission of the pathogenic microorganisms found in environment and on people's hands via food to humans. The present study was undertaken to investigate the microbiological quality of different street food and determining the antibiotic susceptibility of the isolated microorganisms.
Study design: Collection of food samples for isolation of Pathogenic Microorganisms, to identify them by using Biochemical test, molecular test (16sr RNA typing), Antibiotic susceptibility was done by using different antibiotics against the isolates.
Place and Duration of Study: Food samples were collected from street vendors of Sainkpuri area, work was done from December 2020 to April 2021 at Microbiology Department, St. Francis College for Women, Hyderabad.
Methodology: Five samples of street food were collected in sterilized bottles, tested for the presence of microorganisms by following standard microbiological method used for isolation of microorganisms. The organisms were identified by carrying out various biochemical test according to Bergey's Manual of Systematic Bacteriology. The Molecular characterization was done based on 16sr RNA typing. Determining the sensitivity of the isolates against different Antibiotics by employing Kirby Bauer technique.
Results: The organisms isolated from Manchuria and Ragada samples: *Lactobacillus delbrueckii; Lactobacillus casei, Klebsiella pneumonia, Enterococcus faecalis and Vibrio spp* respectively; Samosa and bonda: *Staphylococcus aureus, Lactobacillus delbrueckii and Pantoea dispersa*; Pani puri water: *Staphylococcus aureus* and *Providencia vermicola*. Antibiotic susceptibility tests showed that most of the isolated microorganisms were sensitive to Ciproflaxin.
Conclusion: Hence the quality of street food is found to be low due to following factor: lack hygiene conditions in the food preparation, ingredients may also affect quality of the food, and presence of air borne microorganisms in the surrounding areas of service points.

Keywords: Street food, Quality status, Pani-Puri, Bonda, Samosa, Manchuria, Microorganism

1. INTRODUCTION:

Street food is ready-to-eat food in public place such as market, by a seller often from available food cart. There are a plenty of types of food sold on the street and different places bring different flavors of street foods. The street foods are the well appreciated by consumers,

because of to its taste, low cost, and availability for immediate consumption [1]. The main reasons for spreading of diseases in street food is because sellers do not have sufficient information -and details about food safety. Rise of food originated diseases is related with wrong storage (50%), reheating-storing under inappropriate conditions (45%), and cross contamination (39%). [2]

These street foods are also subjected to cross-contamination from various sources like utensils, knives, raw foodstuffs, flies that sporadically landing on the foods, vendors use their bare hand for serving and occasional food handling by consumers. In most of the cases, tap water is not available for washing hands and utensils at vending sites; hand and utensil washing are usually done in one or more buckets-sometimes without soap [3].

Street food safety remains a major concern in many developing countries, including developed countries like China. Chinese food culture has a very long history of street food. Most cities provide street food for locals and tourists, and street food has become a part of the characteristic Chinese culture. Therefore, street food safety has become a matter of safety concern, and has been shown to be served in poor food handling and unsanitary conditions. The street food vendors are mostly uneducated and often uninformed, and have little effective regulatory or supervisory oversight. In some developing countries, street food has been associated with outbreaks of many foodborne diseases. High levels of coliform bacteria have been found in street food in several countries around the globe, and street food has been identified as a common medium for transmission of antimicrobial-resistant pathogens. [4]

Street vended foods are known to be contaminated with pathogens, which might pose a health hazard. Bacteria belonging to the genus Bacillus, Staphylococcus, Clostridium, Vibrio, Campylobacter, Listeria, Salmonella are reported mostly from street foods. [5]

In this study we have chosen street food like chat, Chinese fast food and deep fry food. In Chat section we have chosen Pani (Pani puri water) of Pani-Puri and Ragada. In the deep fry section, we have chosen Bonda and Samosa. Finally, In Chinese fast food we have chosen Veg. Manchuria.

Different type of pathogenic organisms was identified from the street food, indicating that quality of street food is low and consumption of these kind if street food is not good for health.

2. MATERIAL AND METHODS:

2.1 Collection of samples:

A total number of 5 food samples (Manchuria, Ragada, samosa, bonda and pani puri water samples) were included in this study. Sample was collected from different street vended foods in sainkpuri, samples were collected into sterilized bottles under sterile conditions. All the collected samples were kept on an icebox during transportation to the laboratory and stored at 4°C until testing. They were analyzed within 24h of sampling. The samples were labelled as:

a. FS1- Manchuria
b. FS2- Ragada
c. FS3- Samosa
d. FS4- Bonda
e. FS5- Pani puri water

a b c d e

Fig 1 Samples collected into sterile containers
Samples collected: a. Manchuria, b. Ragada, c. Samosa, d. Bonda, e. Pani puri water

2.2 Microbiological analysis:

One gram of each sample of Manchuria, Ragada, samosa, bonda and pani puri water samples separately inoculated into sterile water and homogenized using glass rod. After that 100µl of homogenized sample was inoculated to nutrient broth and incubated at 37°C for 24h [5]. The overnight culture was streaked into Nutrient agar, Mannitol Salt agar and Eosin Methylene Blue agar**.**

The culture is inoculated into media by spread plate technique and incubated at 37°C for 24h. The colonies on primary cultures were sub cultured by streak plate method on to nutrient agar slants, and incubate at 37°C for 24h. These nutrient slants are preserved for further test.

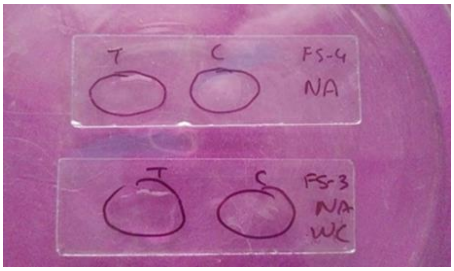
2.3 Auto Vitek method:

It is a fully automated system which performs antibiotic susceptibility testing and bacterial identification. User interface screen for immediate notification of system status to increase productivity. Unique vacuum filler provides both safety and the highest level of automation [6].

2.4 Identification and Biochemical characterization of Isolates:

The isolated colonies were identified by colony morphology and gram staining. The Biochemical test was done based on gram staining, and Bergeys manual was followed for biochemical test. In biochemical characterization endospore staining, acid fast staining, catalase test, oxidase test, carbohydrate fermentation (mannitol and glucose), sodium required for growth test, blood agar and luminescent technique were performed.

S. No	Gram nature	Test performed
1.	Gram positive rod (FS1 NA, FS2 MSA, FS3 NA white colony and growth colony, FS4 NA)	Endospore staining, Acid fast staining, Catalase test, Glucose fermentation, Mannitol fermentation
2.	Gram positive cocci (FS2 NA-small colony, FS3 MSA, FS3 EMB, FS5 NA, FS5 MSA (small and big colony), FS5 EMB)	Catalase test, blood agar and Mannitol fermentation.
3.	Gram negative cocci (FS3 EMB and FS5 NA)	Molecular test
4.	Gram negative rods (FS2 EMB, FS2 NA big colony)	Oxidase test, Glucose fermentation, Sodium required for growth test and Luminescent technique



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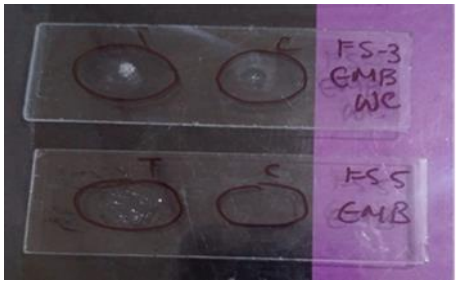

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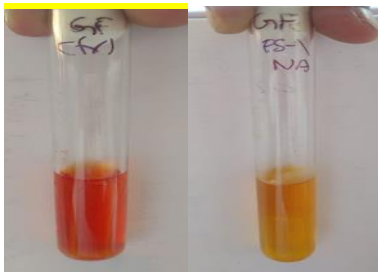

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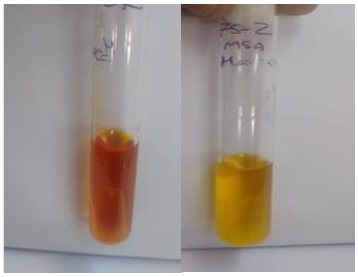

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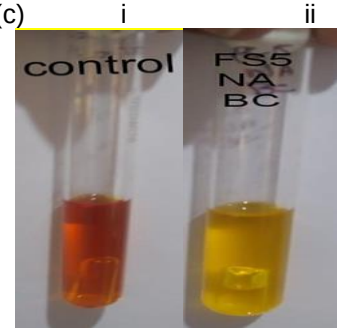

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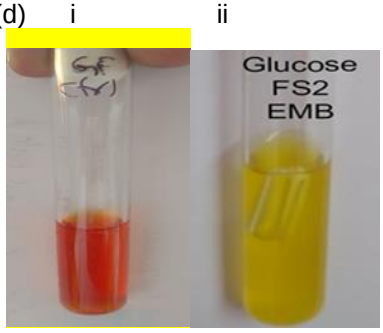

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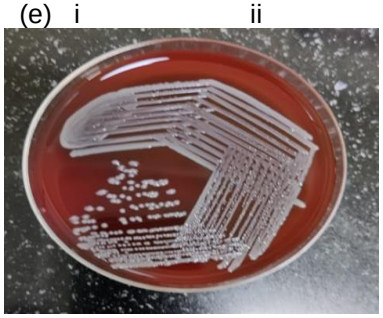

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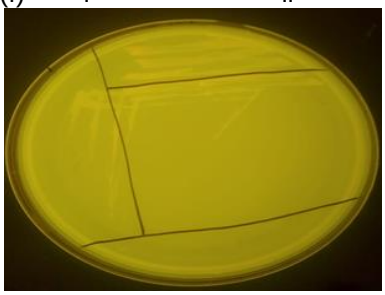

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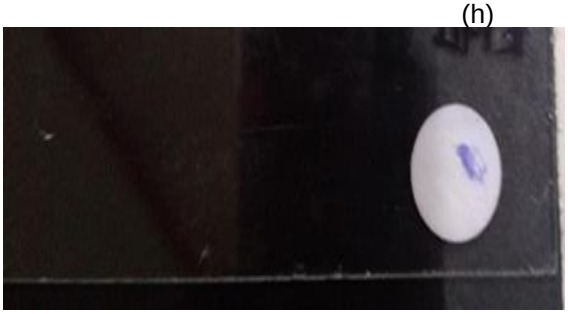

(i)

Fig 2: represents the biochemical test performed to identify the organism:
a. Catalase test: Negative for sample FS4 NA; Negative for sample FS3 NA (White colony),
b. Catalase test: positive for sample FS3 EMB and FS5 EMB,
c. Glucose Fermentation Test: i) control; ii) Positive for the sample FS1,
d. Mannitol Fermentation Test: i) control; ii) Positive for sample FS2 MSA,
e. Mannitol Fermentation test: i) control; ii) Positive for sample FS5 NA,
f. Glucose Fermentation test: i) control; ii) Positive for sample FS2 EMB,
g. Blood agar: Gamma hemolysis of sample FS2 NA,
h. Luminescent Technique: Sample FS2 EMB showing negative for luminescent technique,
i. Oxidase test: Positive for sample FS2 EMB.

2.5 Molecular test (16sr RNA typing)

Traditional methods to identify and characterize microbes require growth of isolates on culture plates. Although these methods have allowed many essential discoveries, there are limitations. In particular, some microbial species do not grow readily in routine laboratory culture conditions or as monocultures, leading to underestimations of the diversity of microbial communities. (Jay-HyunJo1Elizabeth, A. Kenned et al, 2016)

Next-generation sequencing technologies have allowed direct and comprehensive sequence-based interrogation of microbial communities, complementing culture-based methods.

The 16S ribosomal RNA (rRNA) gene is conserved among prokaryotes with specific variable regions that can be used for taxonomic classification, making the 16S rRNA gene a molecular signature to identify members of bacterial communities.

Procedure that is followed

1. Pellet out 1.5ml of an overnight bacterial culture by centrifugation for 2min at 8000 rpm.
2. Remove the culture medium and discard.
3. Resuspend the pellet thoroughly in 180µl of lysis solution and add 20µl of RNase
4. solution, mix and incubate for 2min at RT (15-20 o C)
5. Add 20µl of proteinase k solution, mix and incubate for 30min at 55oC.
6. Add 200µl of lysis solution, vertex thoroughly (15 sec) and incubate at 55oC for 10min.
7. Add 200µl of Ethanol (96-100%) to the lysine and mix thoroughly by vertexing for 15seconds.
8. Load lysine in Hi-elute maniple spin column, centrifuge at 6500xg for 1min. Discard the flow through liquid and place the spin column in a new 2ml collection tube.
9. Add 500µl of prewash solution to the Hi-elute miniprep spin column and centrifuge at 6500xg for 1min. Discard the flow through liquid and reuse the same collection tube with the column.
10. Add 500µl of wash solution to the column and centrifuge for 3min at maximum speed (14000rpm) to dry the column. The column must be free of ethanol before eluting the DNA.
11. Centrifuge the column for the additional 1min at maximum speed if residual ethanol is seen.
12. Pipette 200µl of the elution buffer directly in to the column without spilling to the slides. Incubate for 1min at RT. Centrifuge at 10000rpm for 1min to elute the DNA

2.6 Antibiotic susceptibility test

An antibiotic sensitivity test is used to help find the best treatment for a bacterial infection.

Procedure that is followed:

1. Inoculate the culture into nutrient broth, incubate at 37 ºC for 24h
2. This 24h active culture is used for antibiotic susceptibility test.
3. Prepare Muller Hinton media and autoclave at 121ºC 15 minutes.
4. Pour into petri plates and allow it to solidify.
5. Inoculate 24h active culture by spread plate technique
6. The antibiotic disc should be inserted into petri plate by use of sterile forceps.
7. Incubate at 37ºC for 24h.
8. After incubation, the growth of bacteria is observed.
9. Areas around the antibiotic disc where no bacterial growth can be seen are known as ‘zones of inhibition.
10. These zones show that an antibiotic has been successful in stopping bacterial growth or killing the bacteria.
11. By measuring the diameter of these zones, we can compare the efficacy of antibiotics and monitor antimicrobial resistance.

2.7 Determination of microbiological quality of Street food

The quality of street food depends upon organisms isolated from the street food, isolated organisms are identified by their morphology, gram staining, biochemical test. Depending on these tests we can identify that isolate organism is pathogenic or not, and it is either effecting the quality of street food or not.

3. RESULTS AND DISCUSSION:

3.1 Collection of samples:

The food samples collected were homogenised and inoculated into nutrient broth, after 24h of incubation inoculated into 3 different media

a. Nutrient agar (NA),

b. Eosin methylene blue agar (EMB),

c. Mannitol salt agar (MSA).

3.2 Microbiological Analysis

After 24h, the culture is inoculated into 3 different media (NA EMB, MSA), incubated at 37°C for 24h, Fig.3 represents the colonies observed on respective media, the colonies were sub cultured into nutrient agar and incubated at 37°C for 24h, the growth on nutrient agar slants was observed, these nutrient slants are preserved for further test.
Eight genera of bacteria were isolated, Lactobacillus delbrueckii, Enterococcus faecalis, Klebsiella pneumonia, Lactobacillus casei, Vibrio species, Staphylococcus aureus, Pantoea dispersa, Providencia vermicola were isolated from respective street food samples.

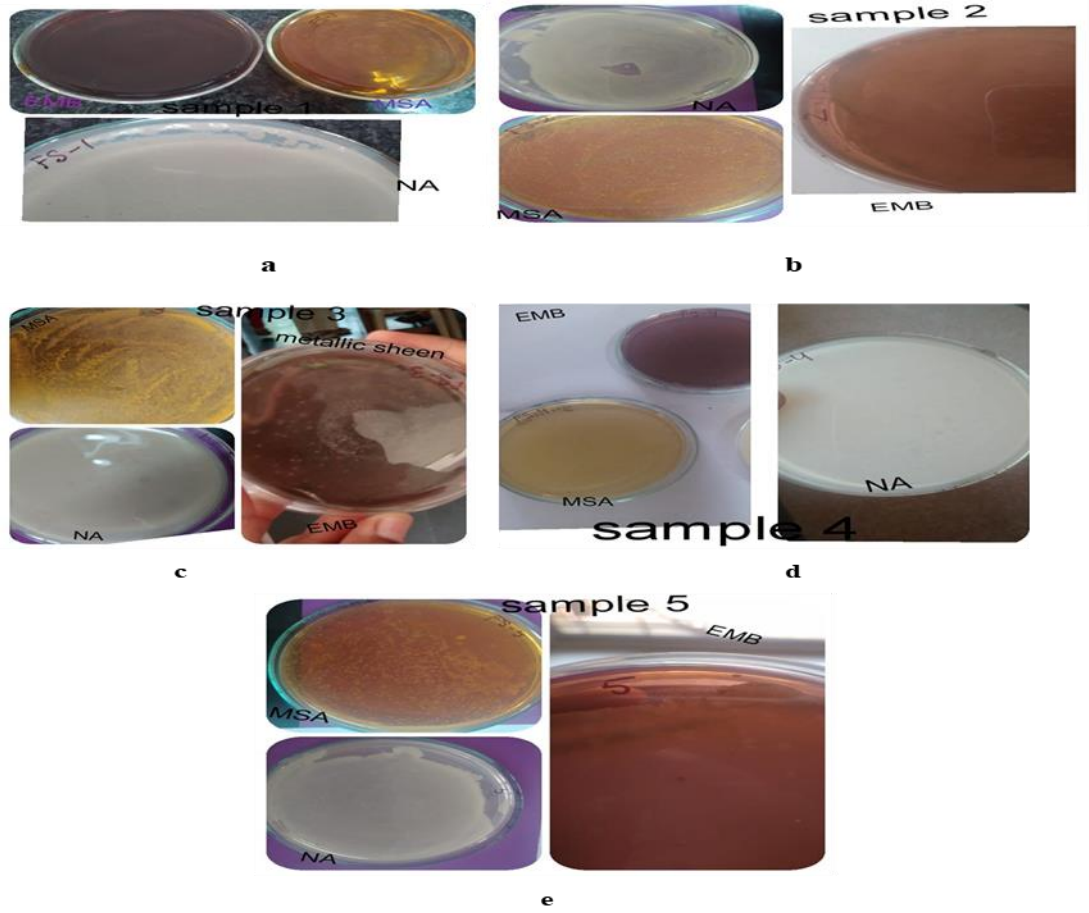

Fig 3 Represents colony characteristics of isolated organisms from street food

a. Sample 1- Manchuria, b. Sample 2- Ragada, c. sample 3- samosa, d. sample 4- Bonda, e. Sample 5- pani puri water.

3.3 Auto Vitek Method

The isolated organism from the Ragada sample is determined as Klebsiella pneumonia by auto Vitek method.

Hence the organisms identified are Klebsiella pneumonia is a bacterium that normally lives inside human intestines, where it doesn't cause disease. But if K. pneumoniae enters into other areas of the body, it can lead to a range of illnesses, including pneumonia, bloodstream infections, meningitis, and urinary tract infections. [20].

The Vibrio spp is a Gram-negative bacterium, several species of which can cause foodborne infection. These bacteria can cause two types of illness: vibriosis and cholera.

3.4 Identification and Biochemical characterization of Isolates and gram staining

In this study, different microbiological techniques were used to identify bacteria isolated from street food samples. The results of cultural characteristics, Gram's staining was used to identify the organisms isolated from Manchuria, Ragada, Samosa, Bonda and Pani-Puri water samples. Gram character and Cultural characteristics of isolated bacteria exhibited on the selective media are represented in table 1.

Table 1: Colony characteristics, morphology and gram staining of the isolated organisms

Sample	Size	Shape	Colour	Opacity	Margin	Elevation	Consistency
FS-1; NA	Medium	Round	Crème	Opaque	Irregular	Flat	Smooth
FS2; NA (Small colony)	Small	Round	Crème	Opaque	Irregular	Flat	Mucoid
FS2; NA (Big colony)	Medium	Round	Crème	Opaque	Irregular	Flat	Mucoid
FS2; MSA	Small	Pinpoint	Yellow	Opaque	Regular	Flat	Mucoid
FS2; EMB			Light purple		Diffused growth		
FS-3; NA (white colonies)	Medium	Round	White	Opaque	Irregular	Flat	Rough
FS-3; NA (growth colony)			White		Diffused growth		
FS-3; MSA	Small	Round	Yellow	Opaque	Regular	Flat	Moist
FS3; EMB (metallic sheen)	Small	Irregular	Metallic sheen	Opaque	Regular	Raised	Smooth
FS-3: EMB (white colonies)	Small	Pinpoint	White	Opaque	Regular	Raised	Smooth
FS4; NA			White		Diffused growth		
FS5; NA (white colony)	Big	Round	White	Translucent	Entire	Flat	Smooth
FS5; NA (small colonies)	Small	Round	White	Translucent	Entire	Flat	Rough
FS5; MSA (small colony)	Small	Round	White	Translucent	Entire	Raised	Moist
FS5; MSA (medium colony)	Medium	Round	White	Translucent	Entire	Raised	Moist
FS5; EMB	Small	Oval	Light violet	Translucent	Entire	Raised	Moist

3.4.1Biochemical characteristics

The organisms isolated from the food samples (FS1, FS2, FS3, FS4, FS5) were characterized by gram staining and further identified based on Bergey's Manual of Systematic Bacteriology.

Below tables represents the biochemical test done based on gram nature:

3.4.1.1 Gram-positive rods

Table -2 Biochemical characterization of Gram-positive rods:

Sample	Endospore staining	Acid fast staining	Catalase	Glucose fermentation	Mannitol fermentation	Organism
FS1 NA	-	-	-	+	-	*Lactobacillus delbrueckii*
FS2 MSA	-	-	-	+	+	*Lactobacillus casei*
FS3 NA(white colony)	-	-	-	+	-	*Lactobacillus delbrueckii*
FS3 NA(growth colony)	-	-	-	+	-	*Lactobacillus delbrueckii*
FS4 NA	-	-	-	+	-	*Lactobacillus delbrueckii*

In current study the organisms isolated from the above samples are *Lactobacillus casei and Lactobacillus delbrueckii* is determined to be the causative microorganism for urinary tract infection. [11] The *Lactobacillus delbrueckii* is a pathogenic organism.

3.4.1.2 Gram-positive cocci

Table-3 Biochemical characterization of Gram-positive cocci:

Sample	Catalase	Mannitol fermentation	Haemolytic pattern on blood agar	Automated Vitek method	Organism
FS2 NA(small colonies)	-	-	Gamma haemolysis	+	*Enterococcus faecalis*
FS3 MSA	+	+	Not required	NR	*Staphylococcus aureus*
FS3 EMB	+	+	Not required	NR	*Staphylococcus aureus*
FS5 NA	+	+	Not required	NR	*Staphylococcus aureus*
FS5 MSA(small colonies)	+	+	Not required	NR	*Staphylococcus aureus*
FS5 MSA(large colony)	+	+	Not required	NR	*Staphylococcus aureus*
FS 5 EMB	+	+	Not required	NR	*Staphylococcus aureus*

In current study organisms isolated from Ragada were *Enterococcus faecalis* which causes nausea, diarrhea, UTI [12] and other isolated organism is *Staphylococcus aureus* causes diseases like Staph food poisoning- it is a gastrointestinal illness, pneumonia and blood stream infections [13].

3.4.1.3 Gram-negative rods

Table 4 Biochemical characterization of Gram-negative rods

Sample	Oxidase Test	Glucose Fermentation	Sodium required for growth	Luminescent technique	Organism
FS2 EMB	+	+	+	-	*Vibrio spp*

Sample	Haemolytic pattern on blood agar	Automated Vitek method	Organism
FS2 NA (large colony)	Gamma Haemolysis	+	*Klebsiella pneumoniae*

Hence the organisms identified are *Klebsiella pneumonia* is a bacterium that normally lives inside human intestines, where it doesn't cause disease. But if *K. pneumoniae* enters into other areas of the body, it can lead to a range of illnesses, including pneumonia, bloodstream infections, meningitis, and urinary tract infections [14].
The *Vibrio spp* is a Gram-negative bacterium, several species of which can cause foodborne infection. These bacteria can cause two types of illness: vibriosis and cholera [15].

3.4.1.4 Gram-negative cocci

Table5. represents samples which were characterized by16s r RNA typing

Sample	16s r RNA typing	Organism
FS3 EMB(metallic sheen)	+	*Pantoea dispersa*
FS5 NA (small colony)	+	*Providencia vermicola*

The isolated organisms from the sample's samosa and pani puri water are determined as *Pantoea dispersa* and *Providencia vermicola* respectively by 16s r RNA typing.

3.5 Molecular detection (16s r RNA typing)

3.5.1 Characterization of aerobic and anaerobic isolate of FS3 EMB and FS5 NA by 16s rRNA typing

- Organism identified as *Pantoea dispersa and Providencia vermicola*

3.5.2 Blast report

BLAST REPORT – FS3

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
Pantoea dispersa strain BJQ0007 chromosome, complete genome	Pantoea dispersa	1602	20022	99%	0.0	99.32%
Pantoea dispersa strain AS18 16S ribosomal RNA gene, partial sequence	Pantoea dispersa	1602	2862	99%	0.0	99.32%
Pantoea dispersa strain S38 ITI 16S ribosomal RNA gene, partial sequence	Pantoea dispersa	1600	2846	98%	0.0	99.32%
Pantoea dispersa strain YBB19B 16S ribosomal RNA gene, partial sequence	Pantoea dispersa	1589	2814	96%	0.0	99.54%

BLAST REPORT- FS5

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
Providencia sp. R4-2A 16S ribosomal RNA gene, partial sequence	Providencia sp. R4-2A	1219	2367	97%	0.0	91.93%
Providencia sp. 1701091 chromosome, complete genome	Providencia sp. 1701091	1216	16615	97%	0.0	92.00%
Providencia sp. 1701011 chromosome, complete genome	Providencia sp. 1701011	1216	16615	97%	0.0	92.00%
Providencia vermicola strain AAUBC-Pv2 16S ribosomal RNA gene, partial sequence	Providencia vermicola	1219	1219	54%	0.0	92.19%

Hence the organisms identified by 16s r RNA typing Providencia vermicola are a gram-negative organism that produce bacterial urease, an important virulence factor associated with the formation of urinary tract stones, the obstruction of long-term urinary catheters, and the development of acute pyel the obstruction of long-term urinary catheters, and the development of acute pyelonephritis [16] ,Pantoea dispersa natural habitat are plant or insect pathogens [17]; although they may also play some beneficial roles, but in some special circumstances such as puncture wounds because of plant matter; contaminated cotton bolls/dressing material, or in the hospital settings, in intravenous fluids, as virulent opportunists; in immunosuppressed and elderly or new-born, this organism grown in the tracheal secretions turned out to be an uncommon, unusual Gram-negative Coccobacillus by the name of Pantoea dispersa [18].

3.6 Antibiotic susceptibility test:

This test will determine the isolated organism is resistant or sensitive to particular drug, hence we determine the drug of choice for the particular organism depending upon on the zone of inhibition. Zone of inhibition is measured in mm.

3.6.1 Antibiotic susceptibility of gram-positive rod:

Table 6 Antibiotic susceptibility test

Sample	AN	ACX	CIP	CLR	CF	LE	CR	CFP	BA	AZ	G	RX
FS1 NA	20	-	27	-	-	20	-	-	17	7	18	12
FS2 MSA	17	-	17	13	-	13	6	13	9	10	15	10
FS3 NA WC	10	-	22	20	-	3	6	13	21	28	15	10
FS3 NA GC	15	-	20	19	-	19	-	13	14	13	14	16
FS4 NA	15	-	20	19	-	19	-	13	7	13	15	15

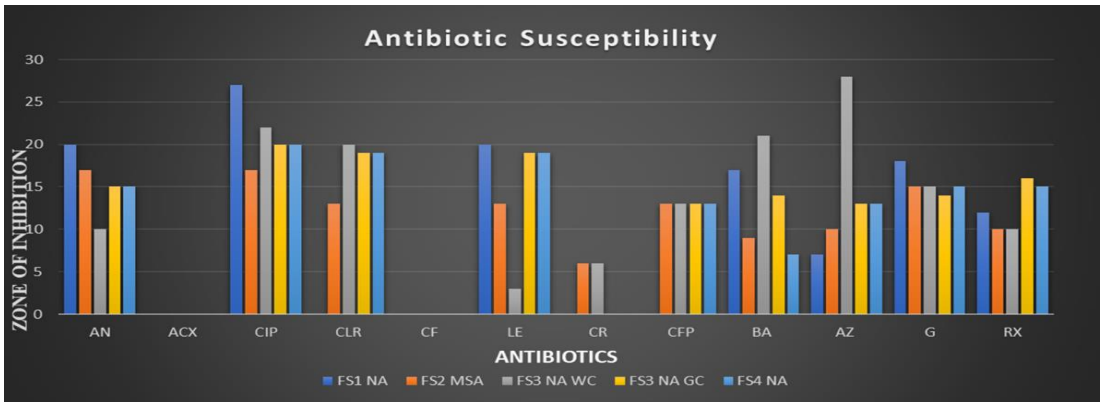

Fig 4 Graphical presentation of antibiotic susceptibility of gram-positive rod

The above Fig 4 represents the graphical presentation of antibiotic susceptibility of gram positive rods which determines that zone of inhibition for sample FS3 NA (white colony) is high for antibiotic **Azithromycin** and zone of inhibition for sample FS1 NA is high for antibiotic C**iproflaxin**, zone of inhibition for sample FS2 MSA is high for antibiotic is amikacin, FS3 NA (growth colony) is having zone of inhibition is high for antibiotic **Ciproflaxin**, FS4 NA having zone of inhibition high for antibiotic **Clarithromycin.** The isolate *Lactobacillus delbrueckii* is resistant **to Ampiclox, cefapoperazone, clarithromycin, Cefuroxime.** The isolate *Lactobacillus casei* is resistant to **Ampiclox and Cefotaxime.**

In current study *Lactobacillus delbrueckii* is resistant to Ampiclox, Cefapoperazone, Clarithromycin and Cefuroxime are drug of choice for the diseases caused by this organism is Ciproflaxin, the isolated organism *Lactobacillus casei* is resistant to both Ampiclox and Cefotaxime, and drug of choice for the diseases that caused by this organism is Ciprofloxacin and Amikacin.

3.6.2 Antibiotic susceptibility of gram positive cocci:

Table 7 Antibiotic susceptibility test

Sample	AN	ACX	CIP	CLR	CF	LE	CR	CFP	BA	AZ	G	RX
FS2 NA (Small colony)	16	-	21	1	17	21	-	17	15	16	14	-
FS3 MSA	20	24	25	19	24	22	24	23	14	15	12	15
FS3 EMB	15	2	1	1	14	18	15	16	15	12	12	-
FS5 NA	17	-	22	2	18	20	13	19	11	13	15	-
FS5 MSA (small colony)	15	17	23	1	18	15	18	17	15	12	13	-
FS5 MSA (Large colony)	17	15	19	7	21	18	16	17	15	12	13	5

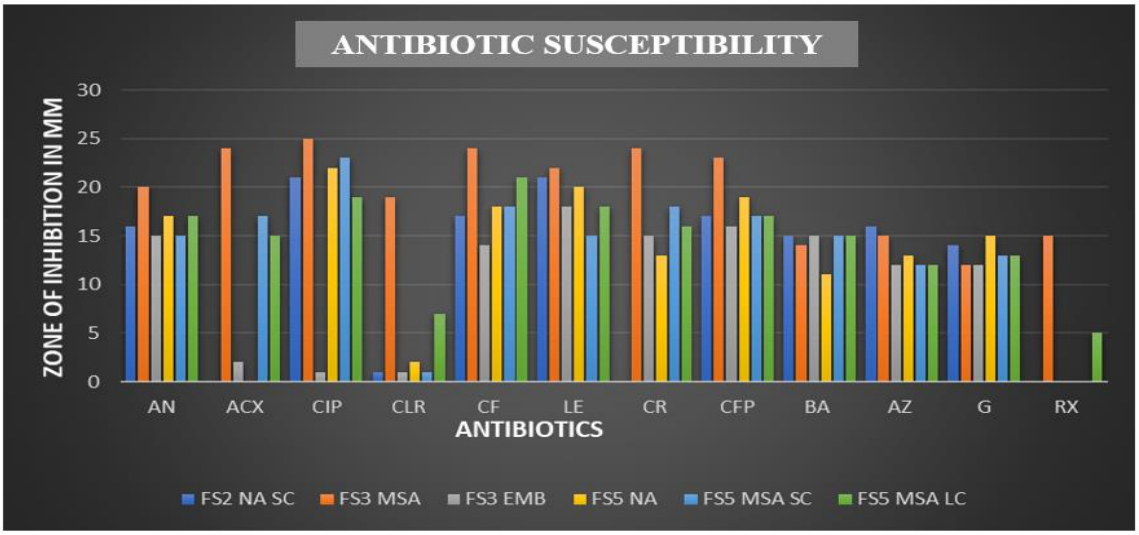

Fig 5 Graphical presentation of antibiotic susceptibility of gram-positive cocci

The Fig 5 determines the zone of inhibition of the isolates, the sample FS2 NA is having zone of inhibition high for antibiotic Ciproflaxin, the FS3 MSA is having zone of inhibition high for antibiotic Ciproflaxin, for FS3 EMB having zone of inhibition high for antibiotic levofloxacin, for FS5 NA having zone of inhibition high for antibiotic Ciproflaxin, for FS 5 MSA (small colony) having zone of inhibition high for antibiotic Ciproflaxin, for FS5 MSA (large colony) having zone of inhibition high for antibiotic cefotaxime. The isolate staphylococcus aureus is resistant to Ampiclox and roxithromycin.

In current study Enterococcus faecalis is resistant Ampiclox, Cefuroxime and Roxithromycin and drug of choice for the diseases caused by this organism is Ampiclox, Cefuroxime and Roxithromycin, and the isolate Staphylococcus aureus is resistant to Ampiclox and roxithromycin and drug of choice for the diseases that caused by this organism is Ciproflaxin.

3.6.3 Antibiotic susceptibility of gram-negative rods

Table 8 Antibiotic susceptibility

Sample	SLB	CTX	CIP	NET	CF	CPZ	AN	CFP	PIT	BA	MF	G
FS2; EMB	-	14	19	15	14	13	16	13	23	10	18	16
FS2 NA big colony	22	25	25	20	21	20	20	29	20	20	18	20

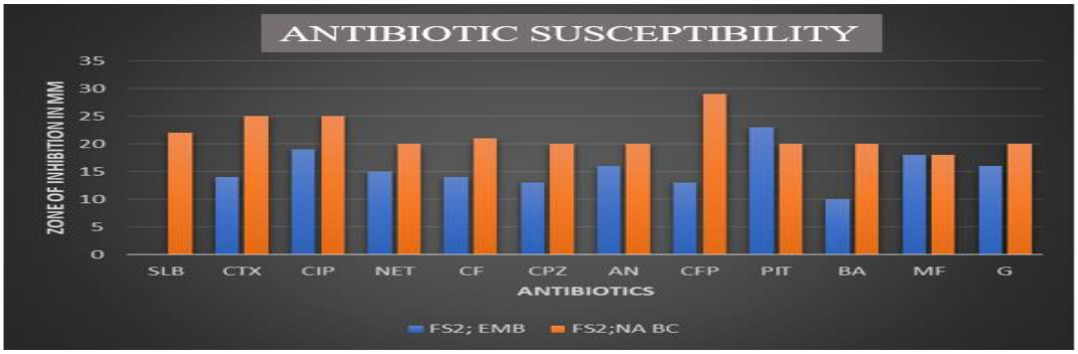

Fig 6 Graphical presentation of antibiotic susceptibility of gram-negative rods

The above Fig 6 represents Graphical presentation of antibiotic susceptibility of gram-negative rods, for sample FS2 EMB zone of inhibition is high for antibiotic Piperacillin and for sample FS2 NA zone of inhibition is high for antibiotic Cefoperazone. The isolate vibrio spp is resistant to Sulbactam and isolate Klebsiella pneumonia is sensitive to all antibiotics.

3.6.4 Antibiotic susceptibility of gram negative cocci

Table 9 antibiotic susceptibility

Sample	SLB	CTX	CIP	NET	CF	CPZ	AN	CFP	PIT	BA	MF	G
FS3 EMB	20	26	35	25	26	25	30	25	23	15	25	20
F5 NA SC	17	-	20	1	20	16	15	20	17	18	15	1

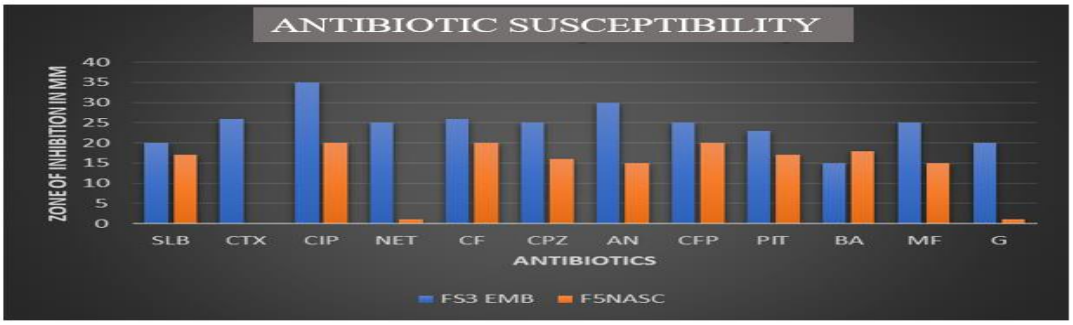

Fig 7 Graphical presentation of antibiotic susceptibility of gram-negative cocci

The above Fig 7 determines the Graphical presentation of antibiotic susceptibility of gram-negative cocci, FS3 EMB having zone of inhibition high for antibiotic **Ciproflaxin,** for FS5 NA having zone of inhibition high for antibiotic **Ciproflaxin, cefotaxime and cefoperazone.** These isolate ***Providencia vermicola*** is resistant to antibiotic **ceftriaxone,** whereas *Pantoea dispersa* is sensitive to all the antibiotics that are used in current study.
In our current study *Pantoea dispersa* has shown sensitive to all antibiotics, research study by [18] states that *Pantoea dispersa* is a multidrug resistant organism.
In current study *Providencia vermicol*a is resistant to Cefotaxime and drug of choice for the diseases that caused by this organism is Ciprofloxacin and Cefoperazone.
Hence the drug of choice will depend on the zone of inhibition, as the zone of inhibition increases the sensitivity of the organism towards particular antibiotic also.

3.7 Determination of the microbiological quality of different type of street food

The FS 1 and 4 which is Manchuria and bonda sample consists *Lactobacillus delbrueckii. Lactobacillus delbrueckii* is determined to be the causative microorganism for urinary tract infection [19].
In FS 2 (Ragada)we have observed organism *Lactobacillus delbrueckii,* pathogenic species which are Vibrio species which causes diarrhea, vibriosis and septicemia, *Enterococcus faecalis* causes diarrhea, nausea and Urinary tract infections infections, *Klebsiella pneumonia* it causes bloodstream infections, meningitis, and urinary tract infections. These pathogens are harmful for our system. Hence, this street food is harmful for consumption.
In FS 3(samosa) we observed *Lactobacillus delbrueckii*, pathogenic species which are *Staphylococcus aureus* it causes diseases like staph food poisoning- it is a gastrointestinal illness, pneumonia and blood stream infections and *Pantoea dispersa* [18] it causes respiratory infections, neonatal sepsis, and bloodstream infections; hence this street food is harmful for consumption.
In FS5 we observed only *Staphylococcus aureus* which causes food poisoning, pneumonia and blood stream infection, *Providencia vermicola* produce bacterial urease, an important virulence factor associated for formation of urinary tract stones. Hence the street food Pani-Puri is harmful for consumption.
These organisms are found and observed by basic testing methods present in our Laboratory. Hence these 5 street foods are analyzed to be not good due to presence of pathogenic organisms as they cause diseases like food poisoning which in severe cases may lead to death, and various illness, hence these street foods are harmful to health.

4. CONCLUSION

The organisms isolated from the different street food samples comprised of Gram-negative rods (Klebsiella pneumonia and Vibrio spp), Gram positive cocci (Staphylococcus aureus, Enterococcus faecalis) and Gram-positive rods (Lactobacillus spp). The organisms are identified by 16s r RNA typing Pantoea dispersa, Providencia vermicola (gram negative cocci). These isolates of street food were identified by biochemical test and molecular test. Antibiotic susceptibility test is done by Kirby-Bauer method, where most of the isolates were resistant to Ampiclox, Cefotaxime, Cefuroxime, Sulbactam. These multidrug resistant foods borne bacteria cause a public health hazard. The microbiological quality of these five-street food is found to be not good due to presence of pathogenic species. The quality of these street food is observed to be poor, due to lack of hygiene conditions while preparing the food, ingredients and the water that are used for preparation may affect quality of the food, due to lack of maintance of the utensils, surrounding area near food preparation, and due to air borne microorganisms.

Hence the microbiological quality of street food can be increased by maintaining hygienic conditions, use of clean water for preparation, preventing the contamination of food by pathogenic organisms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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