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(RESEARCH ARTICLE)

Prevalence and antibiotic susceptibility pattern of *Salmonella spp* isolated from street vended foods in Akola city

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Abstract

Foodborne illness is a major public health problem including India in which the street food prepared and sold by vendors on the street and other public places are a major source of foodborne diseases. *Salmonella* is one of the most important pathogenic genera implicated in food borne bacterial outbreak and diseases. In the present study a total of 55 food samples were collected from various street vended food shops and were used to check the presence of *Salmonella spp*. Results showed that about 40 (73%) samples were found contaminated with *Salmonella spp*. it was found that *S.typhimurium* was most prominently found (37.5%) followed by *S.typhi* (30%), *S.paratyphi* (17.5%) and *S.enteritidis* (15%). All the 40 isolates were checked for the antibiotic susceptibility pattern reveals that most of the isolates showed multiple drug resistance (MDR) which might cause public health hazards if these antibiotic resistance gene transfer to human.

Keywords: Foodborne; *Salmonella spp*; Street vended; MDR

1. Introduction

Salmonella are a group of bacteria that can cause gastrointestinal illness and fever called salmonellosis. *Salmonella* can be spread by food handlers who do not wash their hands or the surfaces and tools they use between food preparation steps, and when people eat raw or undercooked foods. *Salmonella* can also spread from animals to people. People who have direct contact with certain animals, including poultry and reptiles, can spread the bacteria from the animals to food if they do not practice proper hand washing hygiene before handling food. Pets can also spread the bacteria within the home environment if they eat food contaminated with *Salmonella* (FDA, 2019).

Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteraemia and enteric fever (Majowicz *et al*., 2010). *Salmonella* is a rod shaped, gram-negative facultative anaerobe that belongs to the family *Enterobacteriaceae* (Barlow and Hall, 2002). The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori, S. enterica* is the type species and is further divided into six subspecies that include over 2,600 serotypes (Su and Chiu 2007; Ryan *et al*., 2017).

Salmonella serotypes can be divided into two main groups-typhoidal and nontyphoidal. nontyphoidal serotypes are zoonotic and can be transferred from animal to human and from human to human. They usually invade only the gastrointestinal tract and cause salmonellosis, the symptoms of which can be resolved without antibiotics. However, in sub-Saharan Africa, nontyphoidal *Salmonella* can be invasive and cause paratyphoid fever, which requires immediate treatment with antibiotics. typhoidal serotypes can only be transferred from human-to-human, and cause food-borne infection, typhoid fever and paratyphoid fever (Ray and Ryan, 2004).

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Infections associated with *Salmonella* and *Shigella* are among the major public health problems in tropical and subtropical regions of the world. According to a study from the Food and Agriculture Organization (FAO), 2.5 billion people eat street food every day. it is also recognized that street food vendors are often poor, uneducated, and lack knowledge in safe food handling, environment, sanitation and hygiene, mode of food display, food service and hand washing, sources of raw materials, and use of potable water (Hassan *et al*., 2018).

Salmonella typhimurium infection commonly results in symptoms such as abdominal pain, diarrhoea, fever, nausea and vomiting. the bacteria transmitted via ingestion, usually of food contaminated by the feces of an infected person or animal. the incubation period of *Salmonella* can range between 6 to 72 hours, but is more commonly between 12 to 36 hours. There have also been instances of longer incubation period of up to 16 days (Heymann, 2008; Amalie *et al*., 2009).

The street food industry which provides street food in ready –to-eat form are prepared and sold by vendors and hawkers in street and other public places including schools, markets, parks etc are a major source of foodborne diseases. A street food vender is defined as a person who offers food for sale to public without permanent built up structure but a temporary static or mobile stall (Nurudeen *et al*., 2014).

Consumers who depend on such foods are more interested in its convenience and usually pay little attention to its safety, quality and hygiene (Muleta and Ashenafi, 2001). ready-to-eat street foods are also subjected to cross-contamination from various sources such as utensils, knives, raw foodstuffs, flies that sporadically landing on the foods, vendors bare hand serving and occasional food handling by consumers (Muzaffar *et al.,* 2009; Karmaker *et al*., 2018).

In most 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. toilets, waste disposal and refrigeration facilities are rarely available (Hassan *et al*., 2018). Wastewater and garbage are therefore discarded nearby, providing nutrients for insects and other household rodents, which may carry food borne pathogens (Barro *et al*., 2006). the majority of human infections caused by *Salmonella* is related to the ingestion of contaminated foods such as poultry, beef, pork, egg, milk, cheese, seafood, fruit, juices and vegetables (Sattar *et al*., 2014; Hassan *et al*., 2014; Hassan and Ahaduzzaman *et al*., 2016).

The emergence of antibiotic-resistant food borne pathogens has raised the concern of the public as these pathogens are more virulent, causing an increase in the mortality rate of infected patients (Chiu *et al*., 2002). In view of all this the present study was carried out to study the prevalence of *Salmonella spp* among the food samples from the street vended food stalls of Akola City.

2. Material and methods

2.1. Collection of Samples

A total of 55 food samples were randomly chosen and collected from street vendors in the areas of Akola city. These samples were collected in different sterile container to prevent their contact with any other source that can contaminate the samples. Food samples included fast food as well as junk food. All the collected samples were kept in an ice box during transportation to the laboratory and stored at 4 °C until testing. they were analyzed within 2 hours of sampling.

2.2. Preparation of Samples

Adequate amount of different street food samples were uniformly homogenized using a sterile diluent as per recommendation of Balamurugan *et al*., 2013. A quantity of 10 g of each food sample was homoginized with mortar and pestle or blender, the resultant homogenate were aseptically with a sterile spoon added to 5 ml of distilled water and transferred carefully into a sterile test tubes.

2.3. Isolation and Identification of *Salmonella* from food Samples

The isolation and characterization of *Salmonella spp*.were performed based on the Standard procedure described by Cheesbrough (2006). The samples were streaked on Bismuth Sulfite Agar, *Salmonella* and *shigella* agar, and Nutrient agar, and incubated at 37°C for 24 hours for growth of organisms. after incubation isolated colonies were inoculated on nutrient agar slant it was incubated at 37°C for 24 hours and cultures were maintained at 4°C for the further use. The identification of isolates were done by standard conventional methods and compared with Bergey's Manual of Systematic Bacteriology for further identification.

2.4. Determination of Antibiotic Susceptibility/Resistance Pattern of Isolates

To determine the drug sensitivity and resistance patterns of isolated organisms used different types of commercially available antibiotic disc were determined. The antibiotic sensitivity was determined by Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (Difco), according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2007). The pure culture slant colonies were taken and inoculated into nutrient broth and incubated at 37°C for 24 hours after incubation nutrient broth was spread with sterile cotton swab sticks onto sterile Mueller-Hinton agar plates. The antibiotic discs were aseptically placed on the Mueller-Hinton agar (MHA) with a sterile forceps and incubated at 37°C for 24 hours. After overnight incubation at 37°C, the diameter in millimeters of the zones of inhibition around each of the antimicrobial discs was recorded and categorized as resistant, intermediate, and sensitive in accordance with company recommendations (Cappuccino and Carpenter, 2005).

The antibiotics discs were used and their concentrations are as follows: Chloramphenicol (C; 30µg), Ampicillin (AMP; 10µg), Gentamicin (GEN; 10µg), Ceftazidime (CAZ; 30µg), Tertracycline (TE; 30µg), Amoxyclav (AMC; 30µg), Ciprofloxacin (CIP; 5µg), Kanamycin (K; 30µg), Erythromycin (E; 15µg), Meropenem (MRP 10µg), Imipenem-EDTA (IE; 10/750 µg).

3. Results and discussion

In the present study a total of 55 food samples were collected from various street vendors of Akola city (Table 1). The food samples randomly collected from the most of the areas of Akola city and were examined for the presence of *Salmonella spp*. Out of 55 samples about 40 (73%) samples were found to be contaminated with the *Salmonella spp*. (Fig 1). Similarly Eromo *et al*., (2016) also reported about 52% of food samples from street vendors were contaminated with food borne pathogens including *Salmonella spp*. Kibret and Tadesse (2013) also reported 57.5% of *Salmonella spp*. street vended foods. Hasan *et al*., (2018) also reported 60-72.72% of different categories of street food samples were contaminated with *Salmonella spp*. while Tadesse *et al*., (2019) reported 87% of overall prevalence of *Salmonella* and *Shigella*. The 40 isolates obtained were further identified by morphological, cultural and biochemical characteristics. The isolates were probably identified and it was found that *S. typhimurium* was most prominently found (37.5%) followed by *S. typhi* (30%), *S. paratyphi* (17.5%) and *S. enteritidis* (15%) from the different street vended food samples (Fig 2). The present results are in accordance with study of Malik *et al.,* (2015) who also reported isolation of *S. typhi, S. typhimurium* and *S.enteritidis* from dahi balay, fruit chaat and fruit juices from Lahore. Prabhu *et al.,* (2013) reported *S. typhimurium* isolated from raw uncooked coconut chutney from street foods of Thanjavur.Kumar *et al.,* (2017) also reported *Salmonella spp*. from fast food and ice creams from different locations of Tumkur.

Area wise occurrence of *Salmonella spp*. revealed that all the food samples from collected from street vendors of Jawahar nagar, Civil line road, Umri road, New bus stand, Keshav nagar, Gandhi road, Ashok watika G. M. C road, and Satav chowk, of Akola city were 100% contaminated with *Salmonella spp*. While food samples from Ramdaspeth, Kaulkhed and Radhakisan plot area were negative for occurance of *Salmonella spp*. (Table 2).

The *Salmonella spp*. were then subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion technique.the antibiogram of *S. typhi* showed that 100% resistance was recorded for Ampicillin, Erythromycin and Amoxyclave. which was followed by Ceftazidime (91.67%) and Kanamycin (50%) while to Gentamicin, Tetracycline (25%) of isolates showed resistance. to the Meropenem (91.67%) isolates showed sensitivity followed by Chloromphenicol (83.33%) (Fig 3).

The antibiogram of *S. paratyphi* showed that 100% resistance was recorded to Ampicillin, Ceftazidime and Erythromycin which was followed by Kanamycin (42.85%) while to Ciprofloxacin (28.57%). to Chloramphenicol, Gentamicin (100%) isolates showed sensitivity followed by the Tetracycline & Meropenem (85.71%) (Fig 4).

The antibiogram of *S. typhimurium* showed that 100% resistance was recorded to Ampicillin, Ceftazidime, Amoxyclave and Erythromycin. which was followed by Tetracycline and Ciprofloxacin (46.67%) while to Chloramphenicol, Gentamicin, Kanamycin & Imipenem (33.33%) of isolates showed resistance. to the Chloramphenicol (66.67%) isolates showed Sensitivity followed by Meropenem (46.67%) (Fig 5).

The antibiogram of *S.enteritidis* showed that 100% resistance was recorded to Ampicillin, Amoxyclave and Erythromycin which was followed by Ceftazidime (83.33%). while to Chloramphenicol, Gentamicin, Ciprofloxacin, and Imipenem (16.67%) of isolates showed resistance. to the Chloramphenicol (83.33%) isolates showed sensitivity followed by Gentamicin & Imipenem (66.67%) (Fig 6).

Overall many isolates showed multiple drug resistance (MDR). The present study showed that isolates showed higher rate of resistance to the Ampicillin, Amoxyclave, Erythromycin, Ceftazidime. this is similar with the findings of Tadesse *et al*., (2019) who also reported high resistance to Ampicillin (89.5%) while Eromo *et al*., (2016) showed 100% resistance towards Ampicillin and 88.9 % to Chloramphenicol of *Salmonella spp*. In the present study Chloramphenicol showed 80% sensitivity and less resistance exhibited by isolates towards Imipenem, Meropenem, Tetracycline and Gentamicin. These results are in line with the other studies as Kumar and Jangir (2017) showed sensitivity of *S. typhi* towards Imipenem, Tetracycline. Cetinkaya *et al*., (2008) also reported sensitivity of *Salmonella spp*. towards Gentamicin, Kanamycin, Chloramphenicol, Ciprofloxacin but resistance to Tetracycline.

Table 1 Collection of samples from Food Vendors of Akola City

Sr. No.	Samples	Samples Code	Place
1	Noodles	(N)	Jawahar Nagar, Akola
2	Fried Rice	(FR)	Jawahar Nagar, Akola
3	Kachori	(K)	Civil Line Road, Akola
4	Moongvada	(MV)	Civil Line Road, Akola
5	Samosa	(S)	Civil Line Road, Akola
6	Chutney	(C)	Civil Line Road, Akola
7	Jalebi	(J)	Civil Line Road, Inf. Of Bank of Baroda, Akola
8	Panipuri water	(PPW)	Umri Road, Akola
9	Potato Mash	(PM)	Umri Road, Akola
10	Imlie Chutney	(IC)	Umri Road, Akola
11	Rasgulla	(R)	Kedia Plot, Jatharpeth Road, Akola
12	Rasmalai	(RM)	Kedia Plot, Jatharpeth Road, Akola
13	Chocolate Ball	(CB)	Ramdaspeth, Akola
14	Jalebi	(J)	Annabhau Sathe Chowk, Rly Station, Akola
15	Aloobonda	(AB)	New Bus Stand, Akola
16	Pakoda Onion	(PO)	Near Bus Stand, Akola
17	Gudpatti	(GP)	Kaulkhed, Akola
18	Sugarpatti	(SP)	Kaulkhed, Akola
19	Potato Mash	(PM)	Jatharpeth, Infront of Paris Bakery, Akola
20	Pops fry	(PF)	Jatharpeth, Infront of Paris Bakery, Akola
21	Panipuri water	(PPW)	Tapadiya Nagar, Akola
22	Imlie Chutney	(IC)	Tapadiya Nagar, Akola
23	Aloobonda	(AB)	Jai Hind Chowk, Old City, Akola
24	Imlie Chutney	(IC)	Jai Hind Chowk, Old City, Akola
25	Dahibada	(DB)	Jai Hind Chowk, Old City, Akola
26	Panipuri water	(PPW)	Ranpise Nagar, Akola
27	Imlie Chutney	(IC)	Ranpise Nagar, Akola
28	Samosa	(S)	Kedia Plot, Jatharpeth Road, Akola
29	Red Chutney	(RC)	Kedia Plot, Jatharpeth Road, Akola
30	Dhokla	(D)	Kedia Plot, Jatharpeth Road, Akola

31	Imlie Chutney	(IC)	Kedia Plot, Jatharpeth Road, Akola
32	Jalebi	(J)	Ranpise Nagar, Akola
33	Motichur Laddu	(ML)	Ranpise Nagar, Akola
34	Sevpuri	(SP)	Keshav Nagar, Akola
35	Badam Shake	(BS)	Jai Hind Chowk, Old City, Akola
36	Anjeer Shake	(AS)	Jawahar Nagar Square, Akola
37	Panipuri water	(PPW)	Jai Hind Chowk, Old City, Akola
38	Mawa Jalebi	(MJ)	Tilak Road, Malipura, Akola
39	Besan Barfi	(BB)	Ramdaspeth, Akola
40	Besan Papdi	(BP)	Annabhau Sathe Chowk, Rly Station, Akola
41	Mysore Pak	(MP)	Jatharpeth, Umri Road, Akola
42	Kachori	(K)	Tilak Road, Akola
43	Chanachoor	(CC)	Radhakisan Plot, Akola
44	Aloobonda	(AB)	Gandhi Road, Opp. MNP, Akola
45	Moongvada	(M)	Gandhi Road, Opp. MNP, Akola
46	Meduvada	(MV)	Ashok Watika, G.M.C. Road, Akola
47	Coconut Chutney	(CC)	Ashok Watika, G.M.C Road, Akola
48	Idli	(I)	Ashok Watika, G.M.C Road, Akola
49	Sambaram	(SB)	Jatharpeth, Umri Road, Akola
50	Chana Curry	(CHC)	Jatharpeth, Umri Road, Akola
51	Poha	(P)	Satav Chowk, Akola
52	Samosa	(S)	Umri Road, Akola
53	Pakoda	(PK)	Umri Road, Akola
54	Bread Pakoda	(BP)	Tapadiya Nagar, Akola
55	Kachori	(K)	Tapadiya Nagar, Akola

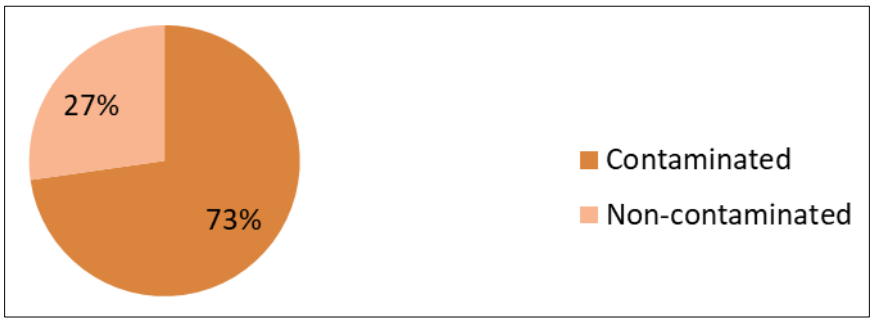


Figure 1 Overall distribution of street food samples contaminated with *Salmonella spp.*

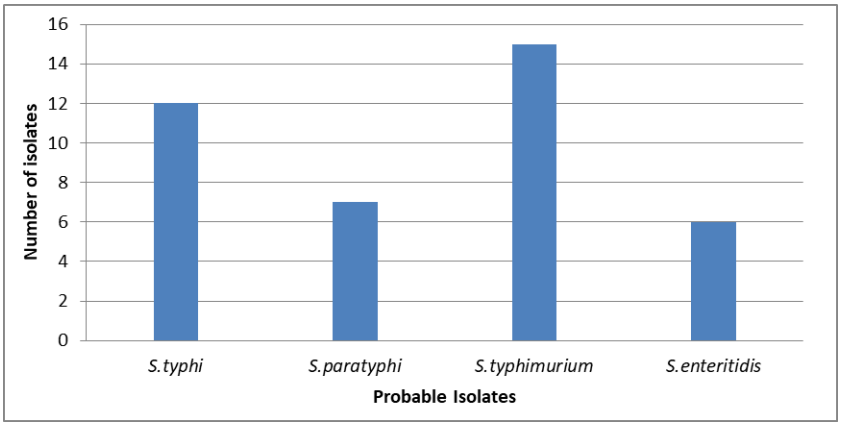


Figure 2 Prevalence of *Salmonella spp*. Isolated from food sample

Table 2 Area wise occurrence of *Salmonella spp*. Among the food samples

Sr. No.	Area	Number of samples collected	Number of samples contaminated with *Salmonella spp.*	Percentage (%)
1	Jawahar Nagar, Akola	3	3	100%
2	Civil Line Road, Akola	5	5	100%
3	Umri Road, Akola	5	5	100%
4	Kedia Plot, Jatharpeth Road, Akola	6	5	83.33%
5	Ramdaspeth, Akola	2	0	0
6	Annabhau Sathe Chowk, Railway Station, Akola	2	1	50%
7	New Bus Stand, Akola	2	2	100%
8	Kaulkhed, Akola	2	0	0
9	Tapadiya Nagar, Akola	4	3	75%
10	Jai Hind Chowk, Old City, Akola	5	3	60%
11	Ranpise Nagar, Akola	4	3	75%
12	Keshav Nagar, Akola	1	1	100%
13	Tilak Road, Malipura, Akola	2	1	50%
14	Radhakisan Plot, Akola	1	0	0
15	Gandhi Raod, Opp. MNP, Akola	2	2	100%
16	Ashok Watika, G.M.C. Road, Akola	3	3	100%
17	Jatharpeth, Umri Road, Akola	3	1	33.33%
18	Satav Chowk, Akola	1	1	100%
19	Jatharpeth, Infront of Paris Bakery, Akola	2	1	50%

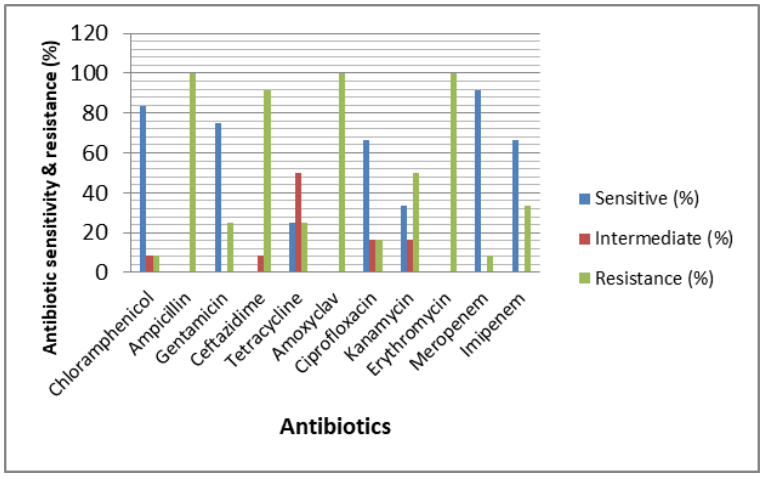


Figure 3 Antibiogram of *S. typhi*

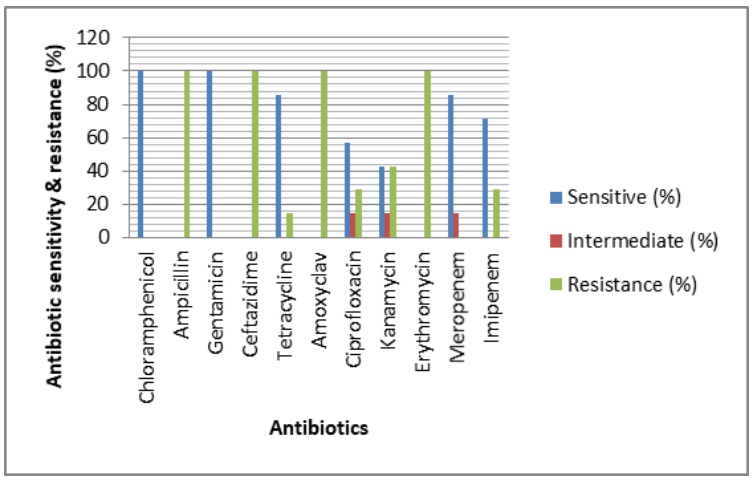


Figure 4 Antibiogram of *S. paratyphi*

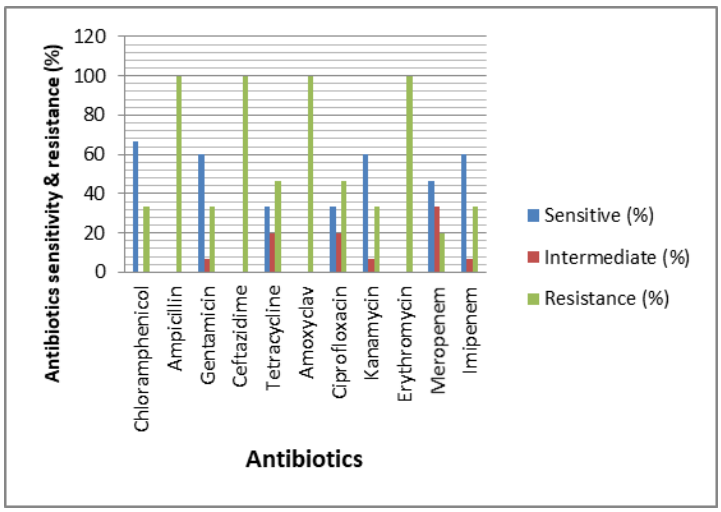


Figure 5 Antibiogram of *S. typhimurium*

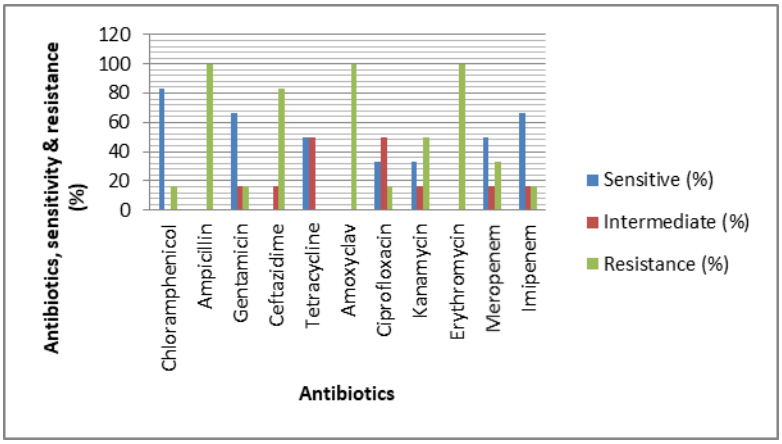


Figure 6 Antibiogram of *S. enteritidis*

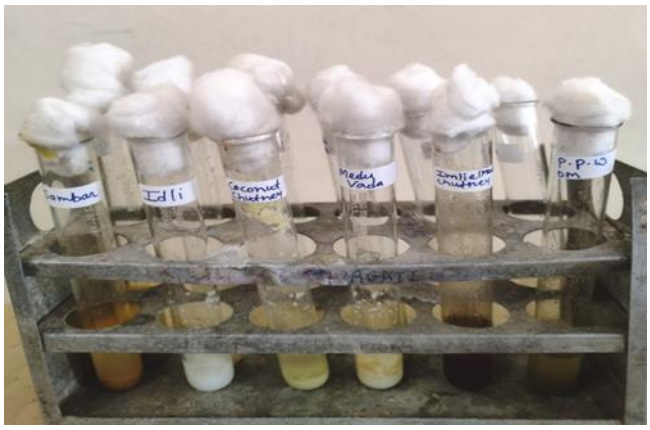
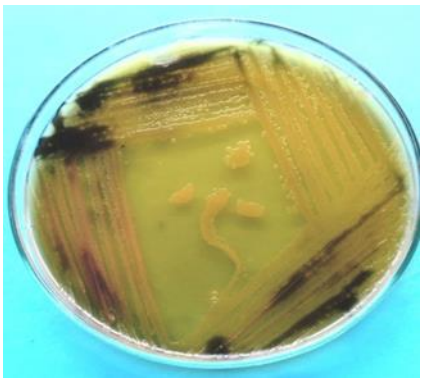
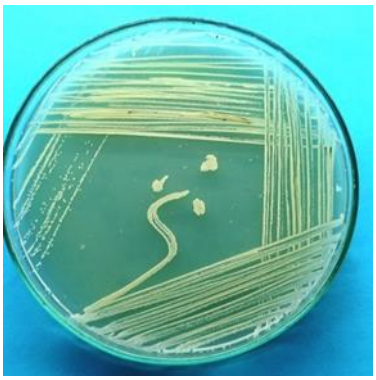


Figure 7 Collection of food samples



On SSA

On BSA



On NA

Figure 8 Growth of Salmonella spp

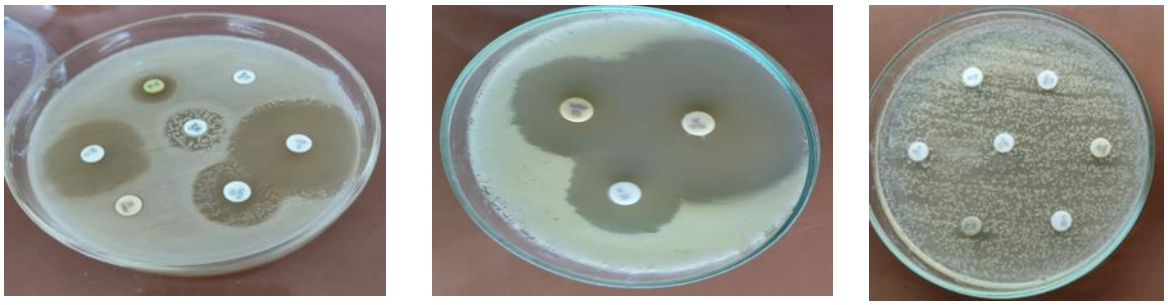


Figure 9 Antibiotic susceptibility/ resistance pattern of isolates

4. Conclusion

In the present study high prevalence of *Salmonella spp.* were observed among the street vended food samples as about 73% of samples showed contamination with *Salmonella spp*. The occurrence of *S.typhimurium* was most prominent followed by *S. typhi, S paratyphi and S. enteritidis*. Most of the areas of Akola city showed considerable presence of *Salmonella spp*. among the street vended food samples. Further the antibiotics resistance among the isolates for many antibiotics was also detected. These findings showed that street foods might pose to serious problem to public health. It would be suggested that awareness amongst street vendors and consumers about food borne pathogens and diseases and safety majors about sanitation by local authorities might reduces the possible outbreaks related to such contaminated food samples.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have no conflict of interest.

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