Isolation and Identification of Pathogenic Bacteria from Various Food Samples of Akola District

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Abstract:

The street food market has expanded quickly in emerging nations such as India. Street food sellers cook and sell food for direct human consumption on streets and in public areas, eliminating the need for additional preparation. In present study 20 samples were taken from different street food, restaurants and hotels in Akola district, Maharashtra. Microbiological analysis was performed. In all the samples bacterial growth was observed. Bacteria isolated include *Salmonella typhi* (3)*, Escherichia coli* (11), *Klebshiella pneumonia* (5), *Serratia marcescens* (1), *Staphylococcus aureus* (2), *Lactobacillus* (5). *E. coli, K. pneumoniae, S. typhi, Serratia, Lactobacillus, S. aureus* showed resistant to ampicillin sensitive to streptomycin and tetracycline.

Key words: Food Samples, Pathogenic bacteria, Antibiotic resistance, Salmonella typhi, Serratia marcescens

INTRODUCTION

The street food industry has grown rapidly in developing countries like India. Street food are prepared and sold by vendors on streets and other public places for direct human consumption without need for further preparation. The rules on food safeties are not followed by street vendors, some hotels and restaurants. The popularity of street food increasing day by day in India due to cheap price and easily availability and more convenient in urban places (Akhilan *et al.,* 2020).

Most type of street food is generally prepared in the open. There is a chance of contamination of the food with dirty water. It is very common to see street food stalls located near open drains especially in developing countries like India. So there is a high chance of contamination with drain water containing faecal matter, in which diarrhoea causing coliform bacteria can be present. Moreover the water used for preparing street food is not filtered and contain contaminants and there is a chance of contracting water-borne diseases like cholera and typhoid. Also street vendors are not washing utensils properly or water used for washing utensils is contaminated it also increase the chance of infection. The raw material used for preparation is placed in open, it also increases chances of food infection through flies and other insects.

Street vendors selling fruit juice should not be consumed as they are generally prepared outside and served later on there by leading to contamination by infectious pathogens. Other utensils like serving glasses are not properly clean or clean with contaminated water.

Small restaurants and street vendors do not have refrigeration and food items kept at room temperature for too long may attract pathogenic micro-organisms that could lead to food poisoning which is extremely dangerous. All food contains small amount micro-organisms; some are beneficial some are pathogenic in nature. Bacteria, virus, parasitic protozoa, toxins, etc. causes food poisoning and can be transferred from one food to another by using same knife, cutting board or other utensils without washing the surface or utensils in between uses.

A food that is fully cooked can become re-contaminated if it touches other raw food or dripping from raw food that contain pathogen. Most of the bacteria present in the food are difficult to control them and some time it reaches a level that can cause death of an individual (Ahmed *et al.,* 2008). The population of Akola district is 2521330 in 2023 (estimated as per aadhar uidai.gov.in 2023 data). The popularity of street food and outdoor food increasing day by day in Akola district, so there is chances of getting infection through consumption of street food.

There are various food-borne pathogenic micro-organisms which causes food infection to humans. Most common and harmful food-borne pathogenic micro-organism is bacteria. Bacteria grow more rapidly on food other than other micro-organisms because replication time of the bacteria is less than other micro-organism. Some pathogenic bacteria like *E. coli* replicate in less than 20 minutes. Food poisoning bacteria grow best at temperature between 5^0C to 60^0C and street food are generally placed in open which can cause food poisoning. The moisture in the food helps bacteria to grow faster. There are some bacteria which cause food-borne illness like *S. typhi, E. coli, K. pneumoniae, S. marcescens, S. aureus, Shigella, Pseudomonas*, etc. More than 90% of the cases of food poisoning are caused by bacteria.

Salmonella typhi is a gram negative bacterium that is responsible for typhoid fever and has been a burden on developing countries. According to WHO report in 2000, typhoid fever was estimated to cause 21.7 million illnesses and 216,000 deaths globally. There are several reason for contamination of street food with *S. typhi*, which include the utensils that enhance cross contamination, vending sites that are filthy in nature, tap water used in preparation of food, waste and garbage produced are discarded nearby that attracts the rodents and insects which may carry food borne pathogens, flies that land on food sporadically and finally handling of food by vendors with bare hands (Tambekar *et al.,* 2009).

Klebshiella pneumoniae is not only a major hospital acquired pathogen but also an important food-borne pathogen that can cause pneumonia, septicaemia, liver abscesses and diarrhoea in human (Othman, 2015). It is normally found in human and also in human and animal faeces. Improper deposition of sewage can lead to contamination of the soil with *K. pneumoniae* and hence it has been found in vegetables, raw meat, fish, milk, etc.

Escherichia coli bacteria normally found in intestines of healthy peoples and animals. Most types of *E. coli* are harmless but few strains such of *E. coli* can cause severe stomach cramps, bloody diarrhoea and vomiting. The most common way to get an *E. coli* infection is by eating contaminated food (Edeh, 2012). According to WHO each year in the United States *Escherichia coli* infection cause approximately 265000 illness and about 100 deaths.

Serratia is also bacterial pathogen which causes food infection to humans, *Serratia* is a gram negative bacterium belonging to the family Enterobacteriaceae. It is widely known as an insect pathogen and as a food spoilage microorganism. The presence of Serratia in food confirms food infection.

Staphylococcus aureus is also one of the major food born pathogen. Man's respiratory passage, skins and superficial wounds are common sources of this bacteria. When *S. aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although cooking destroys the bacteria, the toxin produce is heat stable and may not be destroyed. Staphylococcal food poisoning occurs most often in foods that require hard preparation. Sometimes these types of foods are left at room temperature for periods of time, allowing the bacteria to grow and produce toxin (Edeh 2012). Good personal hygiene when handling foods will keep including bloodstream infection, conjunctivitis, pneumonia, urinary tract infection meningitis, etc. (Wagner, 2001).

Some bacteria are showing antibacterial resistance. Some bacteria developed resistance to antibiotic that were once commonly used to treat them. For example, *S. aureus* is a gram positive bacteria responsible for skin infections and also shows toxic shock syndrome. Several antibiotics were given against the *S. aureus* infections but eventually, the prevalence of multidrug resistance of *S. aureus* strains (MRSA) were very common which causes nosocomial infections (Banerjee *et a*l., 2020).

As much as 70% of the diarrheal diseases in developing countries are believed to be of food-borne origin (Alharbi *et al.,* 2019). The present study was carried out to found total pathogenic bacterial load from street food, restaurants and hotels in Akola district, Maharashtra. Food borne diseases are major problem in developing countries because lack of knowledge about hygiene and lack of personal hygiene. The present study based on pathogenic bacteria from food with special emphasis on *Salmonella typhi, Escherichia coli, Klebshiella pneumoniae, Serratia marcescens, Clostridium botulism, Staphylococcus aureus, Shigella, Pseudomonas*, etc.

MATERIAL AND METHODS

The present work isolation and identification of pathogenic bacteria from food samples was conducted prospectively in the P.G. Department of Microbiology, Ghulam Nabi Azad college of Arts, Commerce and Science, Barshitakli, Dist.-Akola from August 2022 to February 2023. In assessment to isolate and identify pathogenic bacteria from food sample and study their susceptibility and resistance pattern with various antibiotics, present work was undertaken.

Study area:

The study was carried out in Akola city. Akola is Municipal Corporation in Vidharbha zone of Maharashtra. It is spread over an area of approximate 15 sq.km. The current estimated population of Akola city in 2023 is 586000 (WWW.census2011.co.in). There are 23 prabhags which include 76 wards in the town. There are large number of street vendors, hawkers and stall on wheel who sell potables and edibles.

Selection of sampling site:

The area if research in Akola have been classified into:

A. Locality A: **Street vendors:** The samples were collected from street vendors of different locality.

B. Locality B**: Restaurants and Hotels:** Food samples were collected from different Restaurants   and Hotels

C. Dairy: Different samples of milk and curd were collected from different dairies in Akola.

Collection of samples:

Total 20 food samples and dairy products were collected from different places of Akola. Samples were collected in sterile container containing nutrient broth. Then food samples were transferred to microbiology laboratory of Ghulam Nabi Azad college of Arts, Commerce and Science, Barshitakli, Dist. Akola.

- **Food samples:** Food samples (100gm) were collected from different street vendors, hotels and restaurants which include kaju kari, gravy manchuria, panner bhurji, paneer butter masala, etc.

- **Dairy samples:** 50-100 ml of milk and curd were collected from different dairies like mother dairy, Amar milk and local milkman, etc.

- **Sweet samples**: 50-100 gm of sweets like pedha, kaju katli, ras malai, etc. were collected from different sweet marts of Akola.

Isolation and identification of food poisoning bacteria:

A. Propagation of samples: In each sterilized test tube 10 ml nutrient broth is transferred and samples were inoculated in test tube for 24 hrs at 37°C. Culture medium supports growth of microorganisms.

B. **Plating of enriched samples:** All the samples after enrichment were compared with the control tube. Prior incubation loopful of each enriched culture was taken inoculated on the plates of Nutrient media (Hi-media, Mumbai) agar used for the growth of several bacteria. All the samples were inoculated in the triplicate. After inoculation all the plates were kept for incubation aerobically at 37^0c for 24 hrs.

C. **Cultural characterization (Plating on selective Media):** Colonies of relevant pathogens, with different morphological characters were selected to analyse their cultural properties and inoculated on respective selective media viz. Blood agar, Nutrient agar, EMB agar (Eosine Methylene Blue), MacConkey agar, BSA(Bismuth Sulphide Agar), etc. all plates were incubated at 37°C for 24 hrs. All the suspicious screened colonies were then analysed for their biochemical character viz. Carbohydrate fermentation, IMViC, Amylase, Catalase, Coagulase, Oxidase, Urease, and Haemolysis.

5.   Antimicrobial susceptibility testing:

After identification, the pathogenic bacteria were subjected for antibiogram. The antimicrobial susceptibility testing was done by the agar Disk Diffusion Method as described by NCCLS 2002, and Kirby Bauer disk diffusion method now known as the Clinical and Laboratory Standards Institute (CLSI) (Jain and Kamble, 2017).

Antibiotics used: Ampicillin (Amp 10mcg), Tetracycline (Te 30mcg), Penicillin   (P 10mcg).

Sample Collection from various site

RESULTS AND DISCUSSION

Results showed that all street food samples were contaminated with varying level of bacterial counts.

Table No. 1: Sample Collection

Sr. No	Sample No	Isolated organism	Test	Collection date
1	Sample no.1	A, B	+ve	12-09-2022
2	Sample no.2	A	+ve	12-09-2022
3	Sample no.3	NA	-ve	12-09-2022
4	Sample no.4	A, B	+ve	23-09-2022
5	Sample no.5	A, C	+ve	23-09-2022
6	Sample no.6	C	+ve	03-10-2022
7	Sample no.7	A, C, D	+ve	03-10-2022
8	Sample no.8	NA	-ve	03-10-2022
9	Sample no.9	NA	-ve	15-10-2022
10	Sample no.10	E	+ve	15-10-2022
11	Sample no.11	A, E	+ve	28-10-2022
12	Sample no.12	A, B, E	+ve	28-10-2022
13	Sample no.13	NA	-ve	11-11-2022
14	Sample no.14	B, E	+ve	11-11-2022
15	Sample no.15	A, E	+ve	25-11-2022
16	Sample no.16	A, F	+ve	25-11-2022
17	Sample no.17	A, F	+ve	25-11-2022
18	Sample no.18	NA	-ve	16-12-2022
19	Sample no.19	A, B	+ve	22-01-2023
20	Sample no.20	NA	-ve	22-01-2023

Graph 1 : Frequency Distribution of Isolates
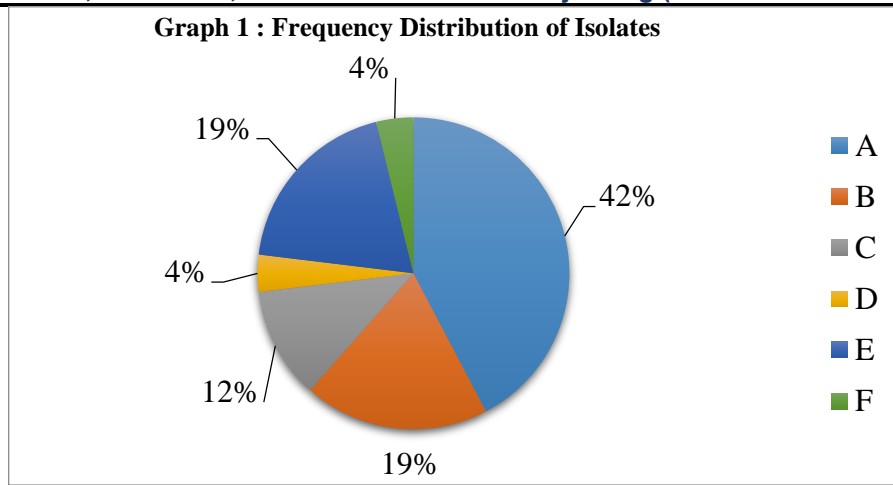
Morphological and cultural characteristic of bacterial isolates

Table 2: Morphological characteristic of bacterial isolates

Sr. no	Character	Bacteria coded as					
		A	**B**	**C**	**D**	**E**	**F**
1	**Size**	1-3 mm	2-4 mm	2-4 mm	2-3mm	1-2 mm	1-3 mm
2	**Shape**	Circular	Circular	Circular	circular	Circular	Circular
3	**Margin**	Regular	Irregular	Irregular	Regular	Regular	Regular
4	**Elevation**	Convex	Convex	Convex	Flat	Flat	Convex
5	**Texture**	Smooth	Hard	Hard	Smooth	Smooth	Smooth
6	**Opacity**	Translucent	Translucent	Opaque	Opaque	Translucent	Translucent
7	**Gram's character**	Gm –ve rods	Gram –ve rods	Gram –ve rods	Gram –ve rods	Gm +ve rods	Gm +ve cocci in bunches
8	**Motility**	Motile	Non-motile	motile	motile	Motile	Non-Motile

Table 3: Cultural characteristics of bacterial isolates

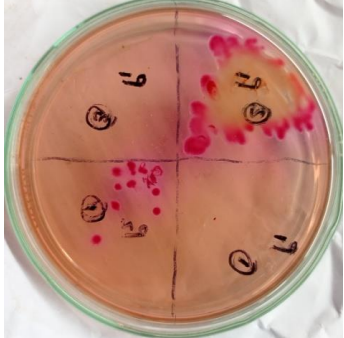| Sr. No	Media	A	B	C	D	E	F
1	NA	Off- white	White	Off-white	Orange coloured	Off-white	Cream coloured
2	EMB	Greenish metallic sheen	-	-	-	-	-
3	MAC	Pink	Pink Mucoid	-	-	-	yellow
4	BSA	-	-	Black	-	-	-
5	MSA	-	-	-	-	-	Golden yellow
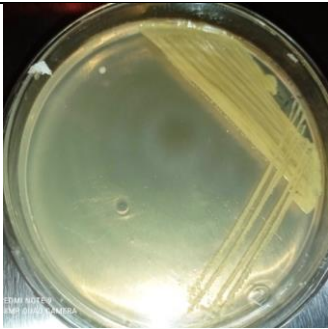
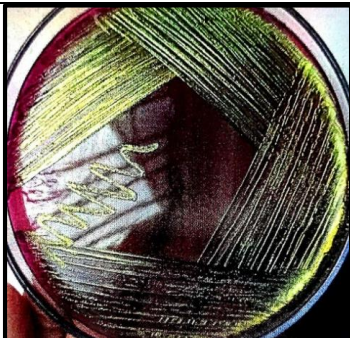NA -Nutrient agar   EMB- Eosine Methylene Blue Agar

MAC- MacConkey Agar   BSA- Bismuth Sulfite Agar

MSA- Mannitol Salt Agar

Morphology of Isolates

|	
S. aureus on Nutrient Agar	*Lactobacillus spp* on Nutrient Agar
K. pneumoniae on MacConkey Agar	*S. typhi* on Bismuth Sulphite Agar
Serratia spp. on Nutrient Agar	*E. coli* on EMB Agar

Biochemical Characteristics of Bacterial Isolates

Table 4 : Biochemical tests of bacterial isolates

Tests		A	B	C	D	E	F
Sugar							
Glucose	Acid	+	+	+	+	+	+
	Gas	+	+	+	+	-	-
Lactose	Acid	+	-	-	-	+	+
	Gas	+	-	-	-	-	-
Mannitol	Acid	+	+	+	+	-	+
	Gas	+	+	-	+	-	-
Tests		**A**	**B**	**C**	**D**	**E**	**F**
IMViC Test							
Indole		+	-	-	-	-	-
MR		+	-	+	-	-	+

VP	-	+	-	+	-	-
Citrate	-	+	-	+	-	-
Enzyme test						
Coagulase	-		-			+
Catalase	+	+	+	+	-	+
Amylase	+	-	-	-	+	-
Urease	-	+	-	+	-	+
Oxidase	-	-	-	-	-	-
Haemolysis	ϒ	ϒ	β	β	ϒ	α

Antimicrobial susceptibility testing:

Graph 2 : Antimicrobial susceptibility

S. aureus	*Lactobcilus. spp.*
Serratia spp.	*S. typhi*
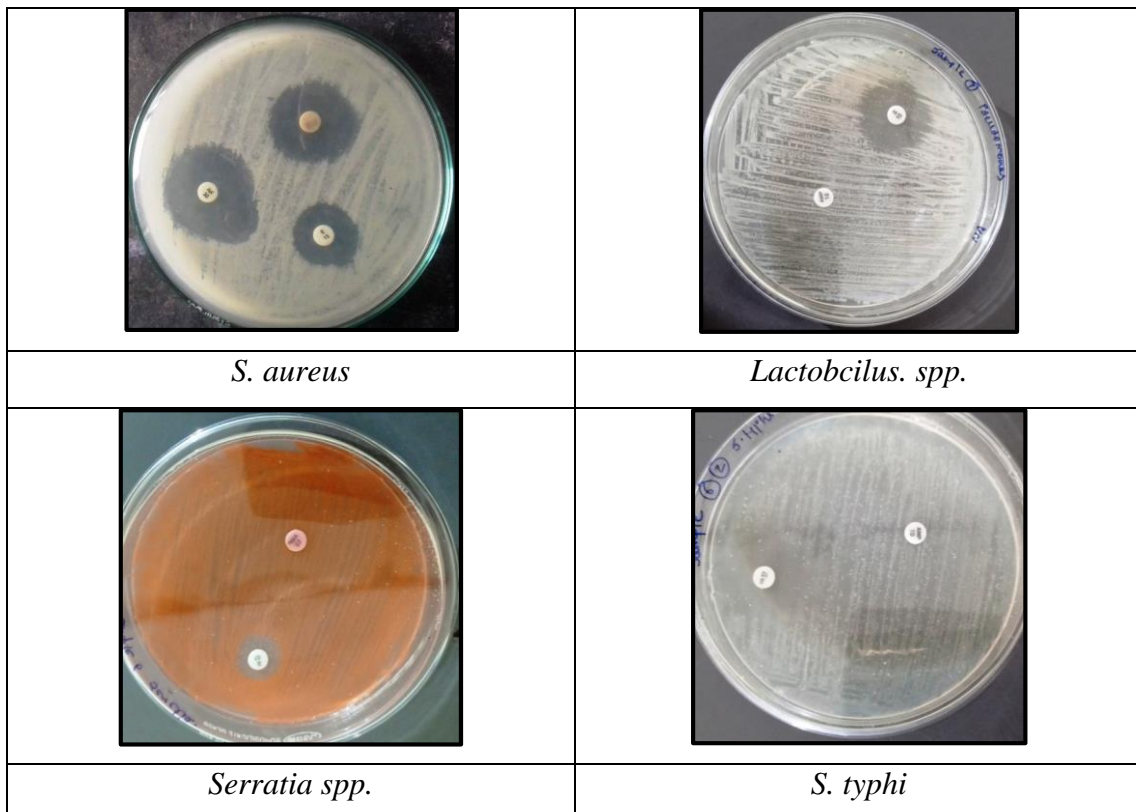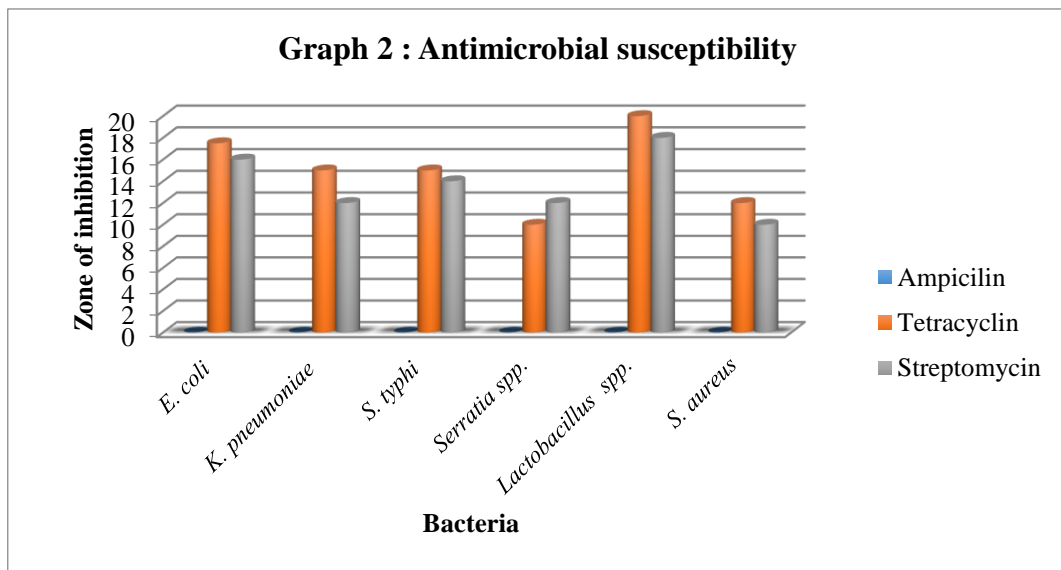
Discussion

Gastroenteritis has remained a major health care problem in India both in terms of human suffering and food-borne illness. The isolation of bacterial in all the food sample (n=20) collected from Akola indicated that the frequency of *E.coli* and *K. pneumoniae* was more significant in street food. The unacceptable total bacterial count was screened from food samples implies extreme contamination and potential health risk of these street food samples. The high incidence of bacterial contamination encountered in this study were mainly due to the largely unhygienic nature of the food preparations and services areas of foods are good indicators of the state of environment in which they are prepared or served. Majority of the street food centres are located beside waste disposal points and duty roads. Furthermore lack of running water, sewage disposal infrastructure, inappropriate storage conditions and the presentation of these food in the open encouraged multiple contaminations. Results showed that isolates gotten from hotel samples shows less pathogenic bacterial count than street food. *Salmonella* spp, isolated in kaju kari are major causes of food borne gastroenteritis and typhoid fever, *Serratia* species were also isolated from curry sample, this may be a result of cross-contamination either from the raw vegetable or water used in the food preparation. *E. coli.* Was isolated in Manchuria is responsible for the high prevalence of diarrhoea, fever, nausea, and cramps in children and adult exposed to contaminated food. *S. aureus* isolated from curry, is a pointer to largely poor personal hygiene, improper storage facilities, use of low quality raw materials and unhygienic environment. The use of the so called food thermo flask to store food before sales are contributed to the proliferation of the bacteria and consequently the high level of microbial count recorded in the study as these device hold foods at bacterial growth temperatures.

In 2016, Hemalata and Virupakshaiah were performed a study to identify various pathogenic bacteria from different sources like; spoiled fruits, vegetables, Dairy products, Bakery products, poultry products and Spoiled rice from local market of Bagalkot district, Karanataka, India. Similarly, in this study food samples were collected in Akola.

In present study 20 samples were taken from street vendors, hotels, restaurants in Akola. Similarly, in 2020, Akilan *et al.,* collected one hundred samples from twenty different street food vendors in Chennai. As a result of their study they found *Salmonella spp. (15), E. coli (25), Staphylococcus aureus (10), Shigella spp. (5), Listeria monocytogenes (5), and other microorganisms (10) among the 60 isolates, Staphylococcus spp.* was most detected isolate (40%) followed by *E. coli (33.3%)* and *Salmonella spp*. (26.7%). Thus their study revealed that street food in Chennai was contaminated with foodborne bacteria that can pose a serious health problem. The present study also revealed that street food in Akola is contaminated with food borne pathogenic bacteria as *E.coli, S.aureus, serratia, S. typhi, K. pneumoniae*.

In 2009, Tambekar *et al.,* collected total of 52 samples of street vended juices in Amravati and analysed. The dominant bacterial pathogen recorded by them was *E.coli* (40%), followed by *P. aeruginosa* (25%), *Salmonella spp.* (16%), *Proteus spp.* (9%), *S. aureus* (6%), *Klebsiella spp.* (3%) and *Enterobacter spp.* (1%). The present study also revealed that *E. coli* is major food borne pathogen in Akola.

In present study all the isolates *viz. E. coli, K. pneumoniae, S. typhi, Serratia, Lactobacillus, S. aureus* showed resistant to ampicillin but found sensitive to streptomycin and tetracycline.

Food hygiene is defined as a sanitary science which aims at producing food which is safe for human consumption and of good keeping quality and this includes any sanitation measures designed to prevent bacteria and other microorganisms of human origin from reaching food stuff. Food hygiene is a subject of wide scope, it aims at studying methods for production and preparation of food, which is safe and of good quality. It covers not only the proper handling of every variety of food stuff and drinks, but also food contact surfaces such as utensils, and apparatus used in the preparation, services and consumption of the food and also the care to prevent contamination with food poisoning bacteria which may originate from the animal or part plant host supplying the food (Umoh and Odibo, 1999).

FACTORS THAT CONTRIBUTE TO FOOD –BORNE ILLNESS

They are, improper cooling of foods, time between preparing and serving, poor personal hygiene, not cooking food properly, Abuse of the time temperature relationship, cross contaminating raw and cooked foods.

- **Poor Personal Hygiene**

Poor personal hygiene can result in food contamination for example when a food personnel, fails to wash hands properly after using the restroom, toilet, is a serious risk of faecal contamination (FDA, 2004). Everyone has bacteria on the skin, mouth, hands and so many other organisms on various parts of the body like hair. Food service personnel can contaminate food and cause food-borne illness. Food workers may transmit pathogens to food from a contaminated surface, from one food to another food or from hands contaminated with organisms from the gastrointestinal tracts. Therefore, hand contact with ready to eat food i.e. food that is edible without washing, cooking or additional preparation by the consumer by the food establishment and that is expected to be consumed in that manner, represents a potentially important mechanisms by which pathogens may enter the food supply (Munide and Kuria, 2005).

- **Abuse of the Time –Temperature Relationship**

Abuse of time temperature relationship is also another factor that can cause food-borne illnesses. To prevent food-borne illness, it is important to control the time that food is in the temperature danger zone. This means hot foods should be kept at 1400F or above and cold foods at 410For below. Don't let cooked or refrigerated foods, such as salads, sat at room temperature for more than two hours (FDA, 2004). Time temperature relationship problems occur because

1. Food is not stored, prepared or held at the required temperature; food is not cooked or reheated to temperature high enough to kill harmful micro organisms

2. Food is prepared in advance of service and proper temperature control is not maintained.

- **Cross-Contaminating Raw and Cooked Food**

Cross-contaminating raw and cooked food is transferring of harmful microorganisms from a surface to food or from one food to another food. Cross contamination can occur when food contact surfaces is not cleaned or sanitized as necessary for food safety. To prevent cross – contamination, it is important to wash hands with soap and warm water before you start preparing food, before you handle a different food (for example, if you just handled raw chicken, wash hands before preparing a salad), and after using the bathroom. Don't sneeze or cough on food. Organisms can "travel" from raw to cooked food, so never let raw food touch cooked food. (FAD, 2004).

CONCLUSION

Street food business has remained largely unregulated in India, notwithstanding the sector contribution to the nation's food security. Wholesome and nutritious street foods have a positive impact on food security, while consumption of street foods of low and below minimum safety standard is injurious to health on an acute or chronic basis. The findings of this study illustrates that bacterial contamination is present in kaju kari, gravy manchuria, panner bhurji, paneer butter masala, pedha, kaju katli in Akola is high, since it is more significant and can cause food poisoning. Pathogenic bacteria isolated such as *E.coli, S.aureus, serratia, S. typhi, K. pneumoniae* which were also isolated in insignificant number could still cause food borne illness depending on the consumer's health status. *Staphylococcus aureus* was less significant in streat food. Therefore, it is very important and necessary for food vendors to always clean and sanitize food contact surfaces, cook and store food properly, so as to reduce the level of food contamination and also to reduce bacterial load to the lowest level, thereby preventing cases of food borne infections. Results also indicated that factors such as the vendors itself (e.g personal cleanness etc), the type of food, have an effect on the bacterial contamination present in foods.

This study showed that *E.coli* and *K. pneumoniae* was major pathogens causing food poisoining to human beings followed by *S.aureus, serratia,* and *S. typhi.*

The antibiotic sensitivity test showed that isolates were highly sensitive to tetracycline and resistant to ampicillin. So, antibiotic tetracycline should be choice of medicine as prior.

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