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ORIGINAL RESEARCH

Isolation and Identification of Pathogenic Bacteria from Ready to Eat Fast Foods from Kothanur, Bangalore.

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Abstract

Due to the use of contaminated foods, foodborne bacteria are viewed as a serious health problem and are becoming a formidable worldwide health complication. Foodborne illnesses are primarily caused by the attractiveness of street food, which is cooked and sold by vendors in an unsanitary environment. In order to identify the biological pathogens in fast food and ascertain their susceptibility to antibiotics, the current observation was made. Street vendors provided samples of their fast food, which were homogenized, serially diluted up to 10-7, and then one millilitre was seeded onto bacteriological media such as MacConkey agar and Blood agar. All of the gathered street food samples had their bacterial pathogens enumerated. The pattern of antibiotic sensitivity showed that Klebsiella was resistant to all of the drugs that were examined. Lack of heat processing processes during preparation and poor personal cleanliness of food handlers may be the cause of tower counts of Klebsiella. These findings highlight the existence of bacteria in street food and the need for better hygienic procedures while preparing food samples.

Keywords

Food safety, Foodborne pathogens, Fast foods, Antibiotic sensitivity

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1. Introduction:

Food safety is a worldwide concern that impacts every person on the earth. Food is currently imported and exported internationally more frequently than in the past due to an increase in international trade and travel. This may lead to the international spread of pollutants and foodborne illnesses. Furthermore, it can be challenging to guarantee that the food we eat is both safe and healthful because food safety regulations might differ significantly between nations. In order to solve this global issue, it is imperative that food safety standards be harmonized between nations and that efficient food safety policies be in place. The term "street fast food" describes food and drinks that are made or sold by street vendors for immediate or later direct human consumption, with or without the need for additional processing or preparation. In today's fast-paced world, ready-to-eat food has grown in popularity and includes a wide variety of options, such as frozen dinners, precut fruits and vegetables, canned soups, and packaged snacks. People may be seriously at risk for health problems as a result of this, particularly in places with inadequate hygiene and sanitation standards. As a result, in order to guarantee that ready-to-eat products are safe for consumers everywhere, it is imperative to spread awareness and take the required steps.

Fateha, 2022 revealed that RTE foods are a serious issue from a public health point of view. To achieve a safer level of E. coli in RTE foods sold for human consumption, public food outlets must improve hygienic and good production procedures. Moreover, MDR E. coli in these foods pose serious public health threats.

Conversely, ready-to-eat (RTE) foods only need to be warmed through; they don't need any further preparation, and they may be consumed either cold or raw without further heating *(Bagumire, 2017).*The modern lifestyle, fast population growth, longer workdays, higher rates of female labour force involvement, and altered cooking and eating customs have all contributed to the rise in RTE food consumption in recent years. RTE foods are convenient for

people who live in busy cities. It is known that handling, preparing, and selling these products might lead to food-borne epidemics *(Oje et al., 2018).*

Furthermore, because they don't need a drawn-out pre-treatment procedure, these meals are flavourful, shelf-stable, reasonably priced, and readily available to consumers *(Spencer, 2005)*. The main situations in which Escherichia coli can provide a health risk are during the preparation and storage of tainted RTE meat. *(Mokhtar and Karmi, 2021)*. RTE meats, however, pose a serious microbiological danger since they have been found to act as carriers of food-borne germs including E. coli *(Kochakkhani, 2016)*. Therefore, a variety of diseases that cause morbidity and mortality globally are classified as food-borne disorders *(Ema et al., 2018)*. Thus, food-traceability systems are absolutely necessary to ensure that final consumers receive safe food and to raise the standard of food processing, particularly for meat and goods derived from meat. *(Sabla et al., 2021).*

A foodborne sickness is an infection or poisoning brought on by eating or drinkIng tainted food or drink. Anybody can be affected by these ailments, regardless of age, health, or lifestyle, and they can range in severity from moderate to severe. *Salmonella listeria* and *Escherichia coli* are the most prevalent foodborne illness kinds, causing symptoms like vomiting, fever, diarrhoea, and stomach pain. Numerous things, including cross-contamination, inappropriate storage, and contaminated equipment, might result in these disorders. To avoid foodborne infections, it is crucial to follow the right food safety procedures, which include cleaning hands and surfaces thoroughly, cooking food to the right temperature, and storing food correctly. Consequently, the purpose of this study was to examine the microbiological quality of a few ready-to-eat foods, particularly those that are frequently consumed by students and include pizza, panipuri, samosas, burgers, and french fries, which are available at all fast-food outlets in Kothanur, Bangalore, Karnataka.

2. Materials and Methods:

2.1. Collection of Samples:

Five ready-to-eat food samples total—pizza, chicken burgers, pani puris, samosas, and french fries - were aseptically gathered at random from various Kothanur eateries and fast-food outlets. The samples were labelled with an identification mark and quickly moved to the proper containers. The samples were handled with extreme caution, placed in an icebox at 4°C, and then brought right away to our lab for examination.

2.2. Sterilisation of Glasswares:

Before and after usage, every item was sufficiently and properly sterilised. Glassware, including pipettes, test tubes, and conical flasks, were carefully cleaned with detergents, adequately rinsed with water, and emptied. For fifteen minutes, the prepared medium and distilled water were autoclaved at 121°C. Before and after usage, metal tools such as the inoculating loop were burned till red. Prior to analysis, the lab bench was always swabbed with 70% ethanol to disinfect it. To lessen contamination of the agar plate tubes, each isolation and inoculation was carried out close to the flame.

2.3 Isolation of Microorganism:

Food samples (Pizza, Burger, Panipuri, Samosa and French fries) were collected and brought to the laboratory for analysis process. Each samples were homogenized separately (10g of sample and 10ml of distilled water). The homogenized samples were taken into 15ml centrifuge tube, samples are centrifuged at 5000rpm for 5minutes. After centrifugation the supernatant was collected and plated onto nutrient agar, potato dextrose agar, Muller Hinton agar. The agar plates are incubated at respective temperatures for 24-48 hours.

2.3.1.Phenotypic characterization

The identification of bacteria is based on their morphological features, which include form, size, margin, and elevation, as well as their cultural traits, which include growth abundance and media color change. These traits are examined on culture plates

2.4 Morphological Identification:

2. 4.1.Gram staining

A single colony of bacteria was aseptically extracted from the food samples using a sterile loop. A tiny smear was created and heat-fixed on a sterile glass slide in a laboratory setting. After adding two drops of Crystal Violet for staining, the mixture was left for a minute. A solution of iodine was added for the washing procedure. Decolorization quickly using acetone, ethanol, or a combination of the two. using safranin as a counterstain. The slide is examined at ten times magnification.

2.5.Biochemical Identification:

The isolates were characterized by the following biochemical tests.

2.5.1.Indole Test

5 drops of kovacs reagent was added directly to the test tube containing microbial broth. Formation of pink to red colour in the top of the medium confirmed the positive result.

2.5.2.Oxidase Test

A piece of filter paper was soaked in 1% Kovacs oxidase reagent and then dried. On the filter paper that has been treated, a loopful of microbial culture is inserted. The positive outcomes are indicated by the color changing from white to violet.

2.5.3. Methyl Red Test

Aseptically inoculate a loopful of microbial culture or inoculum into the sterile nutritional broth, and then incubate for 24 to 48 hours at 37ºC. A few drops of microbial broth were added to a clean test tube, and as an indication, a few drops of methyl red were added as well. Negative findings are indicated by the color yellow, and favourable results are indicated by the color dark red.

2.5.4. Voges Proskauer

Six drops of Voges Proskauer reagent A and two drops of Voges Proskauer reagent B were introduced to the test tube along with a few drops of microbial broth. Positive outcomes are shown by the color cherry red, and negative results are indicated by brilliant yellow.

2.5.5.Catalase Test

A sterile glass slide is taken a drop of hydrogen peroxide is placed on to the slide. A loopful of microbial culture is mixed with hydrogen peroxide. Formation of gas bubbles indicates positive results; absence of gas bubbles indicates negative results.

3. Results & Discussion:

Table 1: *Microbes isolated from the Fast food stuffs*

Sl. No.	Food Stuffs	Locality	Microbes Isolated
1	French fries	Kothanur	*Salmonella spp.*
			Escherichia spp.
			Staphylococcus spp.
2	Pizza		*Salmonella spp.*
			Escherichia spp.
			Escherichia spp.
3	Panipuri		*Salmonella spp.*
			Escherichia spp.
4	Samosa		*Staphylococcus spp.*
			Salmonella spp.
5	Burger		*Staphylococcus spp.*
			Staphylococcus spp.

Five ready-to-eat food samples - pizza, chicken burgers, pani puris, samosas, and french fries were aseptically gathered at random from various Kothanur eateries and fast-food outlets. Four predominant microbes were isolated and observed from the foodstuffs namely - *Escherichia spp., Klebsiella spp., Salmonella spp.* and *Staphylococcus spp.* Their genus level hierarchy were morphologically confirmed using Bergey's Manual of determinative Bacteriology.

Table 2: Phenotypic Characterization (Bergey's Manual of determinative Bacteriology)

Sl. No.	Isolated Microorganisms	Phenotypic Characterization		
		Shape	Elevation	Margin
1	*Escherichia spp.*	Rod	Convex	Fixed
2	*Staphylococcus spp.*	Cocci	Convex	Light
3	*Staphylococcus spp.*	Rod	Low convex	Entire
4	*Klebsiella spp.*	Rod	Dome	Fixed

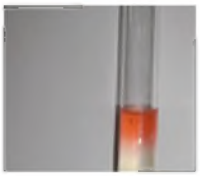
Phenotypic characterization of the isolates such as, shape, elevation and margin were identified using Bergey's Manual of determinative Bacteriology. The phenotype of *Escherichia sp.* was rod in shape, convex with fixed margin. Similarly, cocci shaped, convex light, margin was observed in *Staphylococcus spp.* Rod, low convex and entire margin confirmed the appearance of *Salmonella spp.* Dome elevation, rod shaped, fixed margin was observed for the genus *Klebsiella spp.*

Table 3 :*Morphological & Biochemical Identification*

Sl. No.	Biochemical Tests	*Salmonella spp.*	*Staphylococcus spp.*	*Klebsiella spp.*	*Escherichia spp.*
1	Indole Test	Negative	Negative	Negative	Positive
2	Methyl Red Test	Positive	Positive	Negative	Positive
3	Voges Proskauer Test	Negative	Positive	Positive	Negative
4	Oxidase Test	Negative	Negative	Positive	Positive
5	Catalase Test	Positive	Positive	Positive	Negative

Biochemical tests were performed for the isolated strains, *Escherichia spp.* confirmed the positive response to indole, methyl red and oxidase tests, whereas, negativity were observed in Voges and Catalase. *Klebsiella spp.* confirmed the positive results of voges, oxidase and catalase. Similarly, negative results were confirmed by *Salmonella* and *Staphylococcus spp.* showed negative results in indole oxidase test, whereas, positive in methyl red and catalase.

Table 4: *Biochemical Characterization (Wilson, 2000)*

Microorganisms	Indole Test	Methyl Red Test	Voges Proskauer test	Oxidase test	Catalase Test
Salmonella sp.					
Staphylococcus sp.					
Klebsiella sp.					
Escherichia sp.					

5. Conclusion:

Food safety is a worldwide concern that impacts every person on the earth. Food is currently imported and exported internationally more frequently than in the past due to an increase in international trade and travel. This may lead to the international spread of pollutants and foodborne illnesses. Furthermore, it can be challenging to guarantee that the food we eat is both safe and healthful because food safety regulations might differ significantly between nations. In order to solve this global issue, it is imperative that food safety standards be harmonized between nations and that efficient food safety policies be in place. Therefore, the results of our study can be used to evaluate the public health risk and direct the development of preventative measures that would guarantee the security of street food consumers. These results are frequently used by food safety organizations and regulatory bodies to create policies, guidelines, and awareness-raising initiatives to encourage proper handling and preparation of street food.

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Conflict of Interests:
The authors have no conflicts of interest to declare.

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