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Research article

Zoology

ANALYSIS OF BACTERIOLOGY OF MOST POPULAR STREET FOOD (NOODLES) IN THANJAVUR

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ABSTRACT

This study has demonstrated that the most popular types of food as noodles that are vended on the streets of Thanjavur City do not meet the required quality and safety levels. The emerging needs to be taken to ensure that street are produced and stored hygienically at appropriate temperatures and well protected from flies, dust, wind, and all sources of contamination. The highest bacterial populations were present in nutrient agar plate. In the present study total bacterial density was range from 223 (CFU/g). It was observed that three isolates were gram (-ve) rods and one isolate was gram (+ve) Cocci. Among the four isolates, two were motility rest of them are non-motility. Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. The four isolates were characterized on the basis of biochemical tests. The tests performed to characterize the isolates were Indole, MR, VP and citrate test.

Keywords: Noodles, morphology and biochemical tests.

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INTRODUCTION

The term 'street food' refers to a wide variety of foods and beverages prepared and/or sold by vendors and hawkers especially in streets around trading centers and other public places for immediate consumption or consumption at a later time without further processing or preparation (von Holy and Makhoane, 2006). Whereas most street foods are prepared on a daily basis using a variety of locally available ingredients in order to suit the local tastes and preferences, others are commonly manufactured by local food processing industries, while some may be imported (Mensah *et al.*, 2002). In Uganda, like many other developing countries, street foods provide income and livelihood for many communities particularly the low-income persons. Currently, street food vending in Uganda is probably the single largest employer in the informal sector providing affordable and convenient meals, drinks and snacks to the majority of people especially journeys, those working in urban centers and a high proportion of low-income earners in their residential areas. The majority of people depending on street-vended foods are often more interested in their convenience than safety, quality and hygiene (Kumar *et al.*, 2006; Badrie, 2012).

Microbiological contamination of street foods has become a major public health concern (WHO, 2002). The majority of street food vendors are uninformed of good hygiene practices (GHP) (Mensah *et al.*, 2002), which poses increased risk of contamination for most of the food products involved (Bhaskar *et al.*, 2004; Tambekar *et al.*, 2009). Epidemiological links between street foods such as ready-to-eat (RTE), viz: salad vegetables, sprouts, rice and fish, and disease emergence have been previously reported in India,

The street food plays an important role in meeting the food requirements of urban dwellers in many cities and towns of developing countries including India. The street food feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible. However, food borne illnesses of microbial origin are a major health problem associated with street foods. Keeping this view in the present work was design to assess the selected microbial contamination of food (Noodles) in Thanjavur.

MATERIALS AND METHODS

Sample collection

Approximately 50g of noodles was purchased from street vendors operating in the study area using R.R. Nagar, Bus Terminus, Thanjavur, Tamil Nadu, India. The samples were aseptically transferred into separate sterile stomacher bags, labeled and delivered to laboratory in chilled plastic boxes for analysis within 24hrs of collection (Plate 1). This

sample is in order to determine the foods with microbial contamination.

Inoculation of noodles

The sample was taken in a sterile test tube. 1gram of each sample was aseptically introduced into 9ml of peptone water into test tube; to give 10⁻¹ dilution and spreaded over the nutrient agar medium and the plates were incubated at 37°C for 24 hrs.

Microscopical examination

The morphological analysis of Microorganism was examined by using a sterile loop to pick culture from the culture plate were placed on a microscope slide, covered with a cover slip and observed under the microscope for structure.

Motility test using hanging drop slide

The motility test was performed to differentiate motile bacteria from non-motile one. Before performing the test, a pure culture of the organism was allowed to grow in Nutrient Broth (NB). One drop of cultured broth was placed on the clean cover-slip and was placed inverted over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment of the cover-slip and to prevent evaporation of the fluid by air current. The hanging drop slide was then examined carefully under high power objective (100X) of a compound light microscope. The motile and non-motile organisms were identified by observing motility in contrasting with swinging movement of bacteria.

Bacterial colony counting

100 µl of diluted suspension was poured into the surface of Nutrient agar plate and spread by „L” shaped spreader. The bacteria can thus be isolated and counted by C.F.U i.e. Colony Forming Unit.

$$\text{C.F.U} = \text{No. of colonies/inoculum size (g)} \times \text{Dilution Factor}$$

Morphological characterization of bacterial isolates by Gram staining procedure

The tentative identification of the isolated textile bacteria was done by Gram's staining procedure. The cell shape and Gram's property of bacteria were examined after staining with standard Gram staining procedure. A thin smear of bacterial isolate was prepared on the glass slide, air-dried and heat-fixed. It was stained in the following sequential order: covered with crystal violet for 30 s, washed with distilled water, covered with Gram's iodine solution for 60 s, washed with 95 % ethyl alcohol, washed with distilled water, counterstained with safranin for 30s and finally washed with distilled water. The stained and air-dried slides were examined under microscope using oil-immersion objective technique. Gram-positive bacteria retains the color of crystal violet and stain in purple color, while the Gram-negative takes the color of counter stain

safranin appear pink in color. Biochemical characterization carried out by standard method

RESULTS AND DISCUSSION

Total Bacterial Count

The isolated bacteria was quantified by calculating Colony Forming Unit (C.F.U) i.e. Colony Forming Unit. The obtained C.F.U values are represented in the following table. The numbers of bacterial colonies were isolated by pour plate technique. The highest bacterial populations were present in nutrient agar plate In the present study total bacterial density was range from 508 (CFU/g).

The most basic technique used for classifying bacteria is based on the bacterium's shape and cell arrangement. The most ordinary shapes of bacteria include rod, cocci (round), and spiral forms. Cellular arrangements occur singularly, in series, and in groups. Some species have one to numerous projections called flagella which enable the bacteria to swim and move. *Cocci* or *coccus* for a single cell are round cells, occasionally flattened when being adjacent to each other. *Cocci* bacteria can exist individually, in pairs, in groups of four, in chains, in clusters or in cubes consisting of eight cells. *Bacilli* are rod-shaped bacteria which also can occur individually, in pairs, or in chains (Prabakar *et al.*, 2010; Ying, *et al.*, 2011).

Bacteria classification plays important role in yielding information for disease control. Bacterial species are usually sub-grouped to different types and is used for many crucial pathogenic bacteria such as *Salmonellae*, *E Coli*, and *Vibriones* (Frank, 2009). H.C. Gram in 1884 discovered the Gram stain classification remains an important and useful technique until today. This technique classifies bacteria as either Gram positive or negative based on their morphology and differential staining properties (Prabakar *et al.*, 2010).

Morphology characterization of bacteria

Five isolates were characterized on the basis of colony morphology and the staining characteristics. It was observed that three isolates were gram (-ve) rods and one isolate was gram (+ve) Cocci (plate 2). Among the four isolates, two were motility rest of them are non-motility.

Biochemical Characterization

Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. The four isolates were characterized on the basis of biochemical tests (Table 3). The tests performed to characterize the isolates were Indole, MR, VP and citrate test.

Indole test looks for the presence or absence of tryptophanase enzyme production of the bacteria. If the enzyme is present, it will degrade the amino acid

tryptophan in the media and will produce Indole, ammonia and pyruvic acid. Indole will react with Kovac's reagent to produce a cherry red complex, which indicates a positive indole test. The absence of red color is indicative of tryptophan hydrolysis due to the lack of tryptophanase enzyme.

Methyl Red test detects the ability of microorganism to ferment glucose and to produce acidic end products. Enteric organism produces pyruvic acid from glucose metabolism. Some enteric will then use the mixed acid pathway to metabolize pyruvic acid to other acidic products such as lactic acid, acetic acid and formic acids. This will reduce the pH of the media. Methyl red is a pH indicator which is red at the acidic pH (below 4.4) and yellow at alkaline pH (above 7). The formation of red color after the addition of Methyl red reagent indicates the accumulation of acidic end products in the medium and is an indicative of positive test.

Vogesproskauer test determines the ability of microorganism to ferment glucose. The end products of glucose metabolism, pyruvic acid, is further metabolized by using Butylene glycol pathway to produce neutral end such as acetoin and 2,3 butanediol. When Barrit's reagent A (40% KOH) and Barrit's reagent B (5% solution of alpha naphthol) is added it will detect the presence of acetoin, the precursor in the 2,3- butanediol synthesis. Acetoin in the presence of Oxygen and Barrit's reagent is oxidized to diacetyl, where alpha naphthol act as a catalyst. Diacetyl then reacts with guanidine components of peptone to produce a cherry red colour.

Citrate test determines the ability of microorganism to utilize Citrate. Some bacteria have the capability to convert the salts of organic acids, for example, Sodium citrate to alkaline carbonates. Sodium citrate is one of the important metabolite of Krebs cycle. Certain bacteria use citrate as the sole carbon source. Citrate utilization requires a specific membrane transporter and citrate lyase activity. Citrate is converted to Oxalo acetic acid by citrate lyase and oxaloacetate decarboxylase activity will convert oxaloacetate to pyruvate with the release of carbondioxide. The other products of the reaction are acetate, Lactic acid, formic acid etc. The carbondioxide reacts with sodium and water to form sodium carbonate.

Safety of street foods is questionable as in most cases they are prepared under unsanitary conditions by the vendors who are by and large illiterate and have poor personal hygiene. The chances of contamination of these foods increase greatly due to extremely poor environmental condition in which they are prepared and served. Street foods are the cause of several types of food - borne disease. The water used for drinking and cleaning purposes is often

contaminated due to unhygienic storage and handling. Moreover uses of artificial colours, like metanil yellow, are the cause of serious health hazards. Proper garbage removal facilities are also not available, thus leading to poor environmental condition.

On the basis of morphology and biochemical characterization, the *Escherichia coli*, *Staphylococcus aureus*, *Clostridium* and *Salmonella sp.* were found to be in Noodles.



Plate 1: Bacterial colony

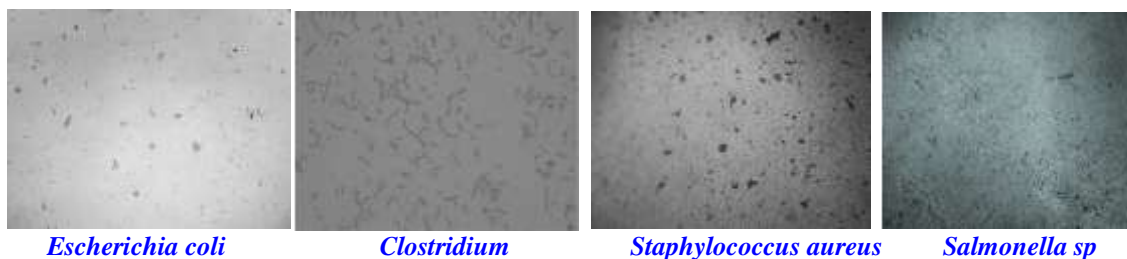
Table 1: bacterial colony counting in infected fish

Dilution	No. of Colonies	Dilution factor	CFU(per g)
10 ⁴	508	10 ⁴	508x10 ⁴

Table 2: Morphological characters of isolated bacterial species

Microorganisms	Morphology	Gram straining	Motility
<i>Escherichia coli</i>	Rod	-	M
<i>Staphylococcus aureus</i>	Cocci	+	NM
<i>Clostridium</i>	Rod	-	M
<i>Salmonella sp.</i>	Rod	-	NM

(+) Positive (-) Negative M= Motile NM= Non motile



Escherichia coli

Clostridium

Staphylococcus aureus

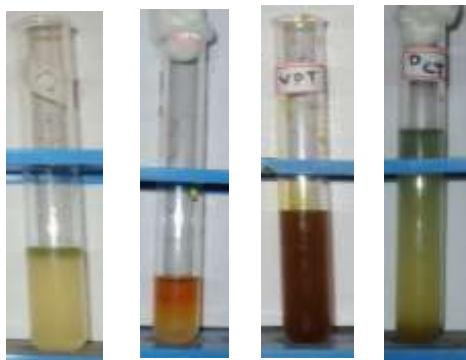
Salmonella sp.

Plate 2: Morphological characters of isolated bacterial species

Table.3: Biochemical characters of bacteria

Microorganisms	Indole test	Methyl Red test	Vogesproskauer test	Citrate test
<i>Escherichia coli</i>	+	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-
<i>Clostridium</i>	+	-	+	+
<i>Salmonella sp.</i>	-	+	-	-

(+) Positive (-) Negative



1) Indole test 2) Methyl Red test 3) Vogesproskauer test 4) Citrate test

Plate 3: Biochemical characters of bacteria

CONCLUSION

The study indicated that the contamination street vended food samples as noodles potentially contaminated with the *Staphylococcus aureus*, *Escherichia coli*, *Clostridium* and *Salmonella sp.* This organism associated with public health hazards. The presence of microorganisms indicated contamination of the processing water as well as the prevailing unhygienic conditions related to the location of the food stalls and especially in dusty road side locations.

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