Isolation and Identification of Bacterial Species Associated with Decayed Commonly Eaten Food Sold in ATAPOLY Restaurants Bauchi, Bauchi State

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Abstract. Isolation and identification of bacterial species associated with decayed, commonly eaten food are paramount to reducing the risk of infection among the populace of the institution. Fifty samples were aseptically collected from the food vendors and subjected to culturing and sub-culturing on nutrient agar. The colonies were then observed for morphological characteristics, followed by biochemical tests and gram staining to ascertain their tentative identity. The results indicated that *Staphylococcus aureus* (37.2%) has the highest occurrence, followed by *Bacillus spp* (18.6%), while Clostridium botulinum has a minor event (2.3%). The presence of these organisms could be attributed to the dirty and unkempt behaviour of the food handlers, which in turn will impose serious health hazards to the immediate community and consumers at large. To reduce the risk associated with these organisms, all personal hygiene measures and the materials involved in the cooking procedures should be sterilised and free from any form of organisms before the cooking process.

Keywords: isolation, identification, bacteria, biochemical, aseptically.

INTRODUCTION

Food is usually composed of carbohydrates, fats, proteins and water that animals or humans can eat for nutrition or pleasure [1]. Food composition is essential for human survival, but along with gaining good nutrition and satisfaction from eating food, humans occasionally consume undesirable biological agents and toxins [2]. Inadequate cleaning of storage and preparation and preparation areas and unclean Kitchen utensils contaminates raw and cooked foods [3]. Contamination can occur even during cooking due to various sources of contamination, and bacteria multiply rapidly if food is kept at room temperature for more than 2 hours. Most bacteria grow undetected because they do not produce an "off" odour or change the colour or texture of the food. Freezing food retards or halts bacteria growth but does not eradicate the bacteria. The microbes can become reactivated when the food is thawed [4].

The current concern in public health is food safety. Consumers are aware of the potential for large-scale food-borne outbreaks because of mishandling or improper food processing and demand a safer supply [5, 6]. The sources of microbial contaminations in the food supply include the environment, food handler, food sources and food itself [7], with kitchen surfaces and taps being the primary sources of cross contaminations [4].

In developing countries such as Nigeria, there are severe concerns about the sanitation of ready-to-eat foods, mainly as portable water is seldom available at preparation sites and fast food stands. Also, most food handlers need basic knowledge of proper personal and environmental hygiene [8].

The research aimed to isolate and identify bacterial pathogens associated with decayed food being solo and eaten at a restaurant within Abubakar Tatari Ali Polytechnic Bauchi, Bauchi State.

MATERIALS AND METHOD

The study area was Abubakar Tatari Ali Polytechnic Bauchi, Bauchi State Nigeria.

A total of fifty samples of the food were collected from the restaurants within the campus. The samples were collected using a sterile cotton wool Swab and then taken to the laboratory for immediate analysis as described by [9].

All materials used were adequately and appropriately sterilised before and after use. Glass wares, such as test tubes, conical flasks, pipettes, etc., were thoroughly washed with detergents, adequately rinsed with water and drained. They were wrapped in aluminium foil and sterilised in a hot air oven at 170 °C for 1 hour. Prepared media and distilled water were autoclaved at 121°C for 15 minutes. Metal equipment, like the inoculating loop, was heated to redness in an open flame before and after use. Before analysis was made, the laboratory bench was always cleaned using 70% ethanol for disinfection. Every isolation and injection was done near the flame to reduce contamination of the agar plate tubes.

The nutrient agar was prepared according to the manufacturer's instructions and Sterilised by autoclaving at 121 °C for 15 minutes. The Swab was then Streaked onto the media and incubated at 37 °C for 24 hours for bacterial growth. Each colony was isolated in a pure form by subculturing in a fresh nutrient agar plate for further studies and identification. Distinctive morphological properties of each pure culture, such as colony form, the elevation of the colony and Colony margin, were observed [10].

The discreet colonies from these subcultured plates were identified by bacteriological analysis using the particular media method described by [11, 12]. Identification and confirmation of Microorganisms Colonial morphology, also termed macroscopic examination for identifying bacterial isolates based on their colony morphology or cultural characteristics (colour, shapes, elevation, margin and pigment formation on the colonies), was done using an experimental microbiology manual. The microscopic investigation included were determination of the bacterial cell wall's gram reaction status (either Gram-positive or negative) after gram staining, aside from examining the cell morphology under the microscope (microscopic morphology). Before the bacterial cell observation under the microscope, gram staining was performed according to the standard gram staining protocol described by [13]. Afterwards, bacterial gram reaction and cell morphology were observed under the microscope with x100 objective lens. On the other hand, the cellular morphology of fungi morphological characterised based on simple staining and staining [10, 12]. Further confirmation of bacteria isolates was achieved by biochemical tests, including Catalase, Coagulase, Indole, Citrate Utilization, Motility and Urease tests to confirm the identified bacteria isolated from the various food samples. Protocols for biochemical analysis were carried out as described by [10, 12].

The present research aimed to isolate and identify bacteria associated with spoiled consumed food sold at the restaurant within Abubakar Tatari Ali Polytechnic Bauchi in Bauchi Metropolis.

RESULTS AND DISCUSSION

A total of 43 Bacterial isolates were recovered. The bacterial isolates were identified and characterised based on their colonial morphology and gram-staining characteristics, as shown in (Tables 1–3).

Table 1 – Morphological Characterisation of the Bacterial Isolates

Isolate code	Colonial Appearance	Morphological Appearance	Gram Staining	Suspected Organisms
AM 1	Dark green and solute colour	Flagella Present	-ve rods	*Salmonella spp.*
AM 2	Red and purple colouration	straight rod-shaped	-ve rods	*E. coli*
AM 3	Irregular flat growth	Rod-shaped motile cells present	+ve rod	*Bacillus spp.*
AM 4	Grey-green with Sunken black cent re	Rod-shaped Bacilli Bacteria	+ve rod	*Listeria monocytes*
AM 5	Gram-positive spore-forming rod	Irregular edges swarming growth	+ve rod	*Clostridium botulinum*

Isolate code	Colonial Appearance	Morphological Appearance	Gram Staining	Suspected Organisms
AM 6	Produce opaque cream Hemolysis	Round resembles that of cocci	+ve rod	*Staphylococcus aureus*
AM 7	Wide zone of beta-hemolysis	Cocci circle	+ve rod	*Pseudomonas fluorescens*
AM 8	Anaerobic rod shape	Swarming motility present	-ve rod	*Proteus mirabilis*

Table 2 – Biochemical characterisation of the recovered bacterial isolates

Sample code	IN	MR	VP	CI	CA	OX	CO	MF	LF	Suspected Organisms
AM 1	-	+	+	-	+	-	+	+	-	*Salmonella spp.*
AM 2	-	-	-	-	+	+	-	-	-	*E. coli*
AM 3	-	+	-	+	+	-	-	-	-	*Bacillus spp.*
AM 4	+	+	-	-	+	-	-	+	-	*Listeria monocytes*
AM 5	+	+	-	-	+	-	-	+	-	*Clostridium botulinum*
AM 6	-	-	-	+	+	+	-	-	-	*Staphylococcus aureus*
AM 7	-	-	-	-	+	-	-	-	-	*Pseudomonas fluorescens*
AM 8	+	+	-	-	+	-	-	-	-	*Proteus mirabilis*

Notes: IN – Indole, MR – Methyl Red, VP – Vogues proskauer, CI – Citrate, CA – Catalase, OX – Oxidase, CO – Coagulase, LF – Lactose Fermentation, MF – Mannitol fermentation.

Table 3 – Number and percentage occurrence of bacterial isolates in spoiled food samples collected

Tentative Organisms	Number of occurrences, n=43	% Occurrence
Salmonella spp.	05	11.6
E. coli	04	9.3
Bacillus spp.	08	18.6
Listeria monocytogens	03	6.9
Clostridium botulinum	01	2.3
Staphylococcus aureus	16	37.2
Pseudomonas fluorescens	03	6.9
Proteus mirabilis	03	6.9

Out of the isolates recovered, 31 were gram-positive bacteria, while 12 were gram-negative. The forty-three bacterial isolates were characterised based on the biochemical test rather than the morphological and colonial features alone.

The results obtained from the research show that the bacteria found in the spoiled food include *Salmonella* (11.6 %), *Escherichia coli* (9.3%), *Bacillus spp* (18.6 %), *Listeria monocytogenes* (6.9%), *Clostridium botulinum* (2.3%), *Staphylococcus aureus* (37.2%), *Pseudomonas fluorescens* (6.9 %) and *Proteus mirabilis* (6.9 %) respectively.

The result also indicates that Staphylococcus aureus (37.2 %) has the highest occurring organisms, while *Clostridium botulinum* (2.3 %) has a minor occurrence. The result agrees with the works of [14], who works on determining and identifying microorganisms responsible for the spoilage of vegetables. The development also concurs with the work conducted by [15], who investigated the microorganisms associated with food spoilage in Uyo Metropolis, Akwa-Ibom, Nigeria, having *Escherichia coli* (28.6%) or the most predominant bacterial isolates.

Staphylococcus aureus in food is a primary bacterial food-borne disease. Its presence in food samples could result from the food handlers' dirty and unkempt behaviour. Symptoms could include vomiting of the bacterial toxin [16].

CONCLUSIONS

Food is essential to the survival of humans. The nutrients obtained from these help in the normal functioning of healthy growth and development of individuals. At the same time, the presence of the bacteria isolated from the research can im-

pose a severe health risk-hazard if proper hygiene, sanitation and measures are not implemented.

To reduce the risk of the outbreak of diseases caused by microbial food contaminations, all measures, such as proper cooking and clean utensils and storage facilities, must be put in place.

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Conflict of interest

The authors declare no conflicting interests.

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