OCCURRENCE OF POTENTIAL BACTERIAL PATHOGENS IN FOOD FROM LOCAL RESTAURANTS IN AZIKORO TOWN, BAYELSA STATE

ABSTRACT

Foodborne gastroenteritis, security and safety is a global concern in today's world, in that regard it is a major concern to public health policies. foods vendor are patronized on daily bases in urban and suburban cities including Nigeria. This study is aimed at investigating the hygienic status of highly patronized food vendors in Azikoro town in Yenagoa, Bayelsa State. Four (4) samples of ready-to-eat street food (rice, yams, beans, and spaghetti) were bought from vendors and brought in chilled to the lab. The samples were analyzed bacteriologically using the streak plate method. The plates were then checked for growth after being incubated for 24 hours at 37°C. In order to subculture, bacteriological agar was used. The growth was then examined, and standard biochemical tests were performed to determine which microorganisms were present. The 40 different colonies in the foods sampled contained nine different bacterial species, with spaghetti having the highest concentration. *Yersinia enterocolitica (*7.5%), *Proteus mirabilis* (12.5%), *Staphylococcus aureus* (7.5%), *Bacillus cereus* (17.5%), *Citrobacter freudii* (5%), *Klebsiella pneumoniae* (30.7692), *Pseudomonas aeruginosa* (7.6923%), *Enterobacter aerogenes* (2.5%), and *Escherichia coli* (20%). The research showed that street foods have the potential to spread bacterial pathogens that cause food-borne illnesses. Consequently, there is a need to create doable plans aimed at ensuring the safety of street food.

Keywords: Food safety, Foodborne, Personal hygiene, Public's health, Vendors

Introduction

Acute gastroenteritis caused by food is a major cause of morbidity and mortality, particularly in developing countries (McLinden *et al*., 2014). The safety of food is a global issue because of the many illnesses associated with contaminated food by foodborne pathogens such as Bacteria, viruses, and protozoa. Foodborne pathogen causes high mortality and morbidity in both the young and elderly. Therefore, there spread and incidence must be controlled in food. In recent years, bacteria like *Salmonella spp., Escherichia coli, Shigella spp., Listeria monocytogenes,* and *Campylobacter jejuni* have been implicated in a number of food outbreaks (Li and Zhu, 2017). The burden of foodborne acute gastroenteritis caused by specific pathogens has been estimated over the last 20 years. However, the global burden of foodborne acute gastro- enteritis remains unknown. (Chen *et al.*, 2018). The emergence of

new foodborne pathogens or the re-emergence of old ones has been attributed to increased industrialization and mass production of agricultural products, globalization of the food supply chain, changes in consumer lifestyle, and related variations in food consumption patterns (Pinu *et al*., 2016).

Additionally, contaminated food can cause disease outbreaks, which would have a negative impact on the economy. A lot of food processing companies recall their products because lab testing was done carelessly. As a result, reliable, quick, and robust methods for detecting pathogens in various food products are required. According to Jasson *et al*. (2010) and Li and Zhu (2017), routine testing in food laboratories continues to rely heavily on time-consuming culture-based identification methods for traditional identification. In contrast, molecular or immunological approaches are quicker and more trustworthy, but they are also more expensive and labor-intensive (Anderson *et al*., 2012). Although there are numerous published works on food sold on the street, no research has been done in this well-known area.

Materials and methods

Study Location

Sample Collection

A total of four (4) street food samples (Rice, Yam, Beans and Spaghetti), were purchased from the food vendors. The samples were transported in ice to the laboratory and studied to determine the hygienic status of each food for potential bacterial pathogens.

Isolation and Identification of Bacteria

The technique used by (Zige *et al.,* 2013) was modified for the purpose of isolating food-borne bacteria. With a sterilized mortar and pestle, five (5g each) samples of each food were homogenized. The resulting homogenate was aseptically added to 9 ml of nutrient broth.

Nutrient agar and Macconkey agar were streaked aseptically from the media straight from the overnight broth culture, and these plates were then incubated at 37°C for 24-48 hours. After incubation, the streaked plates were checked for colonies that displayed distinct cultural traits and subcultured on corresponding media. On nutrient agar, pure colonies were produced by subculturing. Using traditional biochemical techniques, all presumed isolates were further identified (Archana *et al*., 2021; Golden *et al*, 2021; Pujiastuti *et al*., 2018).

Results

In this investigation, a total of 4 street food samples were examined for bacterial contamination. After which the growth was examined, and biochemical tests was carried out to identify the microorganisms present. Nine (9) bacterial species were isolated from the 40 distinct colonies in the foods sampled. Results showed that all the street food samples were contaminated with varying level and types of bacteria. The results obtained are shown in table II, III and IV.

TABLE I: Morphological features of bacteria colonies from the different food samples.

Media	Samples	Morphology of bacterial colonies
MacConkey Agar	Yam	Pale yellow, pink, round
Nutrient Agar	Yam	Pink colonies, Opaque grey, smooth and rough colonies
MacConkey Agar	Rice	Pink, circular colonies
Nutrient Agar	Rice	Pale yellow, white, round
MacConkey Agar	Beans	Pink, rod, round
Nutrient Agar	Beans	White, pale yellow, round, rod
MacConkey Agar	Spaghetti	Pink and yellow, round colonies
Nutrient Agar	Spaghetti	Round, white, pale yellow

The Data in table I above, is used to represent the morphological characteristics of bacterial colonies from all the different food samples before they were subjected to a series of biochemical tests for identification.

TABLE II: Biochemical Test Results

MED	SAM	GL	LA	H_2S	GA	CT	IN	MO	CA	OX	M.ORGANISM
Nut	R1	+	-	-	+	+	-	+	+	+	*Pseudomonas aeruginosa*
Mac	R1	+	+	+	-	+	-	-	+	-	*Citrobacter freundii*
Mac	R2	+	+	-	+	+	-	-	+	-	*Klebsiella pneumoniae*
Mac	Y1	+	-	-	-	-	-	-	+	-	*Yersinia enterocolitica*
Nut	Y1	+	+	+	-	+	-	+	+	-	*Proteus mirabilis*
Nut	Y2	+	+	+	-	+	-	+	+	-	*Citrobacter freundii*
Mac	S1	+	+	-	+	+	-	-	+	-	*Klebsiella pneumoniae*
Nut	S1	+	+	-	-	+	-	-	+	-	*Staphylococcus aureus*
Nut	S2	+	+	-	+	+	-	-	+	-	*Klebsiella pneumoniae*
Mac	S2	+	+	+	-	-	-	-	+	-	*Escherichia coli*
Nut	B1	+	+	-	-	+	-	+	+	-	*Enterobacter aerogenes*
Mac	B2	+	+	-	+	+	-	-	+	-	*Klebsiella pneumoniae*
Nut	B2	+	-	-	-	+	-	+	+	-	*Bacillus cereus*

KEYS: MED= Media used, SAM= Sample**,** GL= Glucose, MOT= Motility, (-) = Negative, CA= Catalase, Nut= nutrient agar media, (+) = positive, IN= Indole, OX= Oxidase, Spag= Spaghetti, CT= Citrate, MED= media, GA= Gas, LA= Lactose, Mac= MacConkey Agar Media.

The Data of Table II above, shows the microorganisms present on each pure isolate from the food samples. They were identified using a series of biochemical tests. Biochemical reactions reveal the vital information necessary for accurately identifying the genera of various bacteria within a sample.

TABLE II: Food samples with their associated bacteria (Pathogens)

Food samples	**Associated bacteria (Pathogens)**
Rice	*Pseudomonas aeruginosa, Citrobacter freundii, Klebsiella pneumoniae*

Beans	*Enterobacter aerogenes, Klebsiella pneumoniae, Bacillus cereus.*
Spaghetti	*Klebsiella pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli.*
Yam	*Yersinia enterocolitica, Proteus mirabilis, Citrobacter freundii*

TABLE IV Percentage occurrence of isolates

Isolates	Number of isolates	Percentage (%)
Pseudomonas aeruginosa	2	5
Escherichia coli	8	20
Klebsiella pneumoniae	9	22.5
Enterobacter aerogenes	1	2.5
Staphylococcus aureus	5	12.5
Citrobacter freundii	2	5
Yersinia enterocolitica,	3	7.5
Proteus mirabilis	3	7.5
Bacillus cereus	7	17.5
TOTAL	**40**	**100%**

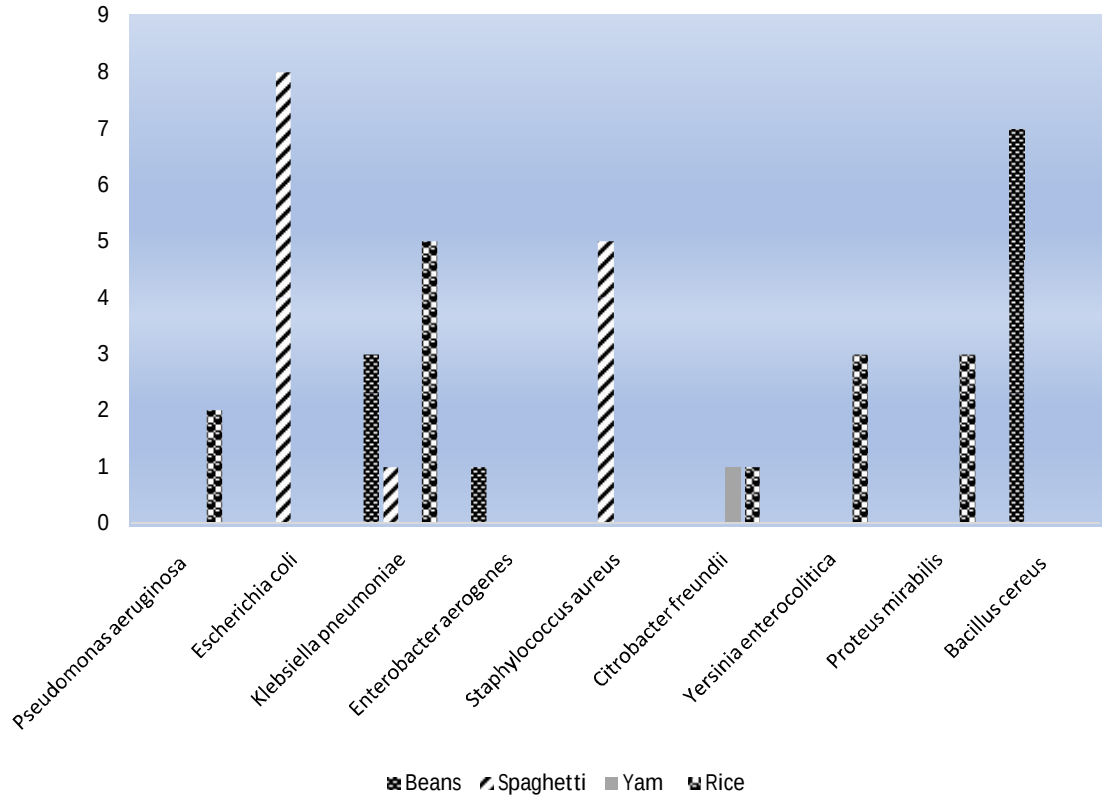

Figure I: Graphical representation of the distribution of the number of each Bacterial isolates from the food samples.

Discussion

Staphylococcus aureus are reported to be 12.5% (Table IV). *Staphylococcus aureus is* a normal component of human and animal skin, *Staphylococcus aureus* is a pathogen that is one of the main causes of food poisoning. Intoxication brought on by *Staphylococcus* enterotoxins is known as *Staphylococcus enterotoxicosis*. According to research done by Pinu *et al* in 2016, it is highly pathogenic and can be found in the air, water, dirt, and excretions. Nausea, vomiting, and diarrhoea are the hallmark signs of staphylococcal food poisoning. 1–6 hours following its consumption Numerous toxins produced by *Staphylococcus aureus*

harm host tissue or boost the virulence of the organism. Because food contamination cannot be completely prevented.

Escherichia coli was isolated from spaghetti and has a percentage of occurrence of 20.00% (Table IV) among the food samples. intestines are typically home to a type of bacteria called *Escherichia coli.* Additionally, some animals' guts contain it. The majority of *Escherichia coli* species are safe and even beneficial to the health of your digestive system. However, some strains can make one sick if consumed in contaminated food or water. While many associate *Escherichia coli* with food poisoning, other types of the bacteria can also cause pneumonia and urinary tract infections. In actuality, *Escherichia coli* is the root cause of 75 to 95 percent of urinary tract infections. (Komala *et al*., 2013)

Escherichia coli, *Staphylococcus aureus,* and *Bacillus* sp. were found to be the most common bacteria contaminating cooked meals, bottled drinks, and fresh fruit in a study on street vended foods in Atbara City in the Naher Elneen state of Sudan (Abdalla *et al*., 2009; Elfaki and Elhakim, 2011; Ameko *et al.*, 2012). Numerous origins point to unsanitary practices in the study area. 17.5% of samples tested positive for *Bacillus cereus* (Table IV) mainly isolated from beans (Table IV). It is a gram-positive bacterium that causes foodborne diseases and is widespread in nature and foods. *Klebsiella pneumoniae* has a Frequency occurrence of 22.55% (Table IV) and it was isolated from Rice, Spaghetti and Beans. (Table IV). It is an opportunistic bacterium found in various microbiological niches such as soil; the skin, intestines, and faeces of mammals and food. *K. pneumoniae* has been documented to cause bacteraemia, pneumonia, and urinary tract infection as researched by Pinu *et al.*, 2016. When found in food, *K. pneumoniae* raises risk to the general public's health, either directly as a pathogen or indirectly as a potential carrier of antibiotic genes that could spread to other pathogens in the microflora of food and the human gut. Table IV shows the frequency of occurrence of *Enterobacter aerogenes*, which was isolated from the beans sample.

Enterobacter aerogenes, a member of the normal flora of the human gastrointestinal tract (Jha *et al.*, 2016). *Pseudomonas aeruginosa* had a prevalence of 5% (Table IV), it is commonly found in the environment, especially in freshwater. Reservoirs in urban communities include hot tubs, jacuzzis, and swimming pools. It can cause a variety of community infections such as folliculitis, puncture wounds leading to osteomyelitis, pneumonia, otitis externa and many others. (Archana *et al*., 2021) *Pseudomonas aeruginosa* is a gram-negative, aerobic, non-spore forming bacterium that can cause various infections in immunocompromised and immunocompromised hosts (Abdolshahi *et al*., 2020) Controlled food samples mean that these street food samples have extremely high levels of contamination and potential health risks. The high rates of microbial contamination encountered in this study were mainly due to the unsanitary nature of the food preparation process, and food service areas are a good indicator of the environmental condition. The majority of street food centres are located next to waste disposal points and public roads. In addition, the lack of potable water, wastewater treatment infrastructure, inappropriate storage conditions and the outdoor sale of these foods have created conditions for many types of food to become contaminated. The use of so-called food warmers to preserve food before sale contributes to microbial growth and therefore increases numbers of bacteria observed in the study, as these devices are unique and Maintain food at a temperature where bacteria grow.

Conclusion and Recommendation

This study illustrates the unsanitary situation of street food due to the presence of potential pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogenes, Klebsiella pneumoniae*, *Bacillus cereus* and *Escherichia coli* in food at different levels. It is therefore recommended that food vendors have all their equipment used for cooking and dispensing be properly cleaned, thus avoiding foodborne infections and

gastroenteritis associated with these pathogens. According to our results, personal hygiene measures should also be applied to sellers and those who regularly patronize them.

References

Abdalla, M.A.; Suliman, S.E and Bakhiet, A.O (2009). Food safety knowledge and practices of street vendors in Atbara city (Nahar Elneel State Sudan). *Afr. J. Biotechnol*. 8(24), 6967-6971.

Abdolshahi, A and Yancheshmeh, B.S (2020). Food Contamination. Pg 1-2.

Anderson, N.W.; Buchan, B.W.; Riebe, K.M.; Parsons, L.N.; Gnacinski, S and Ledeboer, N.A (2012). Effects of solid-medium type on routine identification of bacterial isolates by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Journal of Clinical. Microbiology.* 50, 1008–1013.

Ameko, E.; Achio, S.; Alhassan, S and Kassim, A (2012) Microbial safety of raw mixed vegetable salad sold as an accompaniment to street vended cooked rice in Accra. J. Biotechnol. 11(50), 11078-11085

Angelo, K.M.; Conrad, A.R.; Saupe, A.; Dragoo, H.; West, N.; Sorenson, A.; Barnes, A.; Doyle, M., Beal, J.; Jackson, K.A.; Stroika, S.; Tarr, C.; Kucerova, Z.; Lance, S.l.; Gould, L.H.; Wise, M and Jackson, B.R (2017). Multistate outbreak of Listeria monocytogenes infections linked to whole apples used in commercially produced, prepackaged caramel apples: *Epidemiology and infection*, 145(5), 848–856.

Archana, A.; Swagata, T and Vinay, K (2021). Biochemical tests for detecting hydrogen sulphide producing bacteria. Volume 2, page 53.

Chen, Y.; Yufeng, W.; Jiangen, S.; Baifeng, C.; Shushu, D.; Lei, D and Jiajia, D (2018) The correlation between family food handling behaviors and foodborne acute gastroenteritis: a community-oriented, population-based survey in Anhui, China. *BMC Public Health* 18:1290

Elfaki, A.E and Elhakim, S.A.A (2011). Quality Evaluation of Two Sudanese Street Foods of Animal Origin. *Adv. J. Food Sci. Technol*. 3(3), 219-223

Esha, S.; Dwij, R.B and Binod, L (2009). Occurrence of Salmonella in drinking water samples of urban water supply system of Kathmandu. *Botanica Orientalis – Journal of Plant Science*, 6: 52-55.

Golden, C.E.; Rothrock, M.J and Mishra, A. (2021) Mapping foodborne pathogen contamination throughout the conventional and alternative poultry supply chains. *Poult. Sci.* 2021, 100, 101157.

Jasson, V.; Jacxsens, L.; Luning, P.; Rajkovic, A and Uyttendaele, M (2010). Alternative microbial methods: an overview and selection criteria. *Food Microbiology*. 27, 710–730.

Jha, P.; Kim, C.M and Kim, D.M (2016). Transmission of *Enterobacter aerogenes* septicemia in healthcare workers; 5(1):1397.

Kerr, K.G and Snelling, A.M (2009). Pseudomonas aeruginosa: a formidable and ever-present adversary. *Journal of Hospital Infections.*

Komala, M., Debjit, B and Sampath-kumar K.P. (2013) Urinary Tract Infection: Causes, Symptoms, Diagnosis And It's Management. *Journal of Chemical and Pharmaceutical Sciences*. 6 (1)25-28

Li, H and Zhu, J (2017). Targeted metabolic profiling rapidly differentiates *Escherichia coli* and *Staphylococcus aureus* at species and strain level. Rapid Commun. Mass Spectrom. 31, 1669–1676.

Marollo, R (2016). Microbiology of *Bacillus cereus*, The diverse faces of *bacillus cereus*. page 1-13.

Marshall, K.M.; Nowaczyk, I and Raphael, B.H (2014). Identification and genetic characterization of *Clostridium botulinum* serotype a strain from commercially pasteurized carrot juice. *Food Microbiology* 44: 149–155.

Marušić, A and Glob, J (2011). Food safety and security: what were favorite topics for research in the last decade? 1, 72–78.

Merchant, L.E.; Rempel, H.; Forge, T.; Kannangara, T.; Bittman, S.; Delaquis, P.; Topp, E.; Ziebell, K.A and Diarra, M.S. (2012). Character-ization of antibiotic-resistant and potentially pathogenic Escherichia coli from soil fertilized with litter of broiler chickens fedantimicrobial-supplemented diets. *Can. J. Microbiol.* 58, 1084–1098.

McLinden.; T, Sargeant, J.M.; Thomas, M.K, *et al*. (2014). Component costs of foodborne illness: a scoping review. *BMC Public Health.* 14:509.

Mulcahy, L.R., Isabella, V.M and Lewis, K (2014). *Pseudomonas aeruginosa* biofilms in disease; 68(1):1-12.

Pinu, F.R (2016). Early detection of food pathogens and food spoilage microorganisms: application of metabolomics. 213–215.

Pujiastuti, E.S.; Tarigan, J.R.; Sianturi, E and Ginting, B.B. (2018) The effect of chicken manure and beneficial microorganisms of EM-4 on growth and yield of kale (Brassica oleraceae acephala) grown on Andisol. IOP Conf. Ser. *Earth Environ. Sci.,* 205, 012020.

Zige, D.V., Ohimain, E.I and Mynepalli, S.K.C. (2013). Enteric Bacteria from ready to eat food vended in Amassoama Community in Niger Delta and its health implication. *ISOR Journal of Environmental Science, Toxicology and Food Technology, (*6)4: 62-69.