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Identification and susceptibility Profile of Bacteria Isolate from street Food (Fried Rice)

Asso. Prof. Glory Baysah, Danny Sendolo, Francis O. Somah, Rosetta Saywon, Steven Ekki, and Idris Lateef

Asso. Prof. Glory Baysah, Adventist University of West Africa, baysahg@auwa.edu.lr,
Danny Sendolo, Adventist University of West Africa, sendolodanny@gmail.com
Francis O. Somah, -Adventist University of West Africa, somalf@auwa.edu.lr,
Rosetta Saywon, -Adventist University of West Africa, rosettagono@gmail.com
Steven Ekki,- Adventist University of West Africa, Stevene@auwa.edu.lr
Idris Lateef -Adventist University of West Africa, idrislateef@gmail.com

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Abstract

Rice is one of the most important staple foods for human population worldwide, especially in Africa and the West Indias. In Africa, rice has been used to improve nutrition quality, boost food security, foster rural development and support sustainable healthcare. Fried rice is one of the most popular street food sold and used on several programs in Liberia. In contrast to these potential benefits, street food has been identified as a major public health risk due to lack of basic infrastructure and services and also difficulty in controlling the large numbers of food preparations. This research seeks to identify common food borne bacteria found in fried rice and determine their susceptibility pattern.

Six fried rice samples from different vendor were collected aseptically and were used for this study. Identification of the isolates was done using biochemical test following standard procedure. A total of eight antibiotics, were use in this study. The Antibiotics susceptibility was done using disk diffusion methods.

In this study the result shows that, a total of seven (7) bacteria (3- *Proteus*, 2 – *Citrobacter, 1- Pantoea* and *1- Serratia*) were obtained from fried rice and the percentage occurrences of bacteria were 42.8%- *Proteus*, 28.6% – *Citrobacter, 14.3%- Pantoea* and *14.3% - Serratia*. . The presence of coliform bacteria in almost all food samples may be an indication of unhygienic handling of the food after cooking and or lack of good manufacturing practices which may account for post processing contamination recorded in this study. In this study most of the organism showed highly resistant to the antibiotics used.

This study concluded that street food are potential vector for transferring of pathogenic bacteria and good hygiene practice should be employed and good measures should also be taken to ensure that the occurrence of these organisms in foods is kept within limit to reduce the risk of bacterial contamination. There should be more emphases placed on antibiotic stewardship, infection control, resistance tracking, and diagnostic test development in order to reduce or eliminating pathogens and indicator organisms from food.

Keywords: Fried rice, Bacteria; Pathogen; Disease; Street Food

Introduction

The food and Agriculture Organization (FAO) defined street vended food as ready to eat food and beverages prepare and sold by street vendor for immediate consumption'. In most parts of Africa, street vended foods are very popular medium of dietary needs (FAO, 1991). This mostly informal trade is important socially and economically in meeting much needed food demands in urban areas. In Liberia, there are varying types of street food however fried rice remains the most consumed locally. Fried rice is a typical traditional cuisine in Liberia made with a lightly fried rice marinated with various vegetables and spices cooked in vegetable oil along with barbecued sauce and fried chicken enjoyed during festive season, ceremonies and as street food. It is popular amongst street food consumers because it is a source for inexpensive, nutritious and often convenient menu for cities and urban areas; it also provides much needed employment and income for vendors. Despite street vended food playing an important role both economically and socially in meeting food demands for urban people, microbiological contamination of street food is a global public health problem, which is a significant contributor to the transmission of food borne diseases (Al Mamun M, Rahman SMM, Turin TC, 2013). Food borne diseases causes high morbidity mainly in developing countries; due to poor hygienic condition during food preparation and the lack of awareness about food safety (Saha SK, Saha S, Shakur S, Hanif M, Habib MA, Datta SK, et al. 2009). Many studies have reported, unhygienic practices substantially contributing to the entry of bacterial pathogens to food include contamination from surfaces of raw materials and equipment, improper storage or refrigeration, and unsanitary conditions of surfaces in the working environment. Street foods pose a risk to public health because they are openly displayed and are exposed to dust, insects, and the hands of the food handlers and customers (Venter I, 2005). In areas frequented for street food are places where sanitary facilities are rarely available for workers. Often than not domestic and other waste are disposed off in nearby drainages and roadside, providing nutrients for flies and rodents known carriers of food borne pathogens (WHO, 2018). As a result, ready to eat prepared street foods are commonly exposed to a variety of potential public health risks. Contamination with pathogenic microorganisms like *E. coli, Salmonella, Shigella, Campylobacter and S. aureus* may occur during preparation, post-cooking and at various handling stages. The burden of food borne disease shows that approximately 600 million (1 in 10) people get ill aftereating contaminated food. Food borne diseases cause an estimated 420,000 deaths per

year including 125,000 children under the age of 5 years. According to the IAHO and WHO (2022), food borne diseases morbidity in Africa is estimated at 91 million monthly, and nearly 137,000 deaths. Understanding the bacteriological quality of street foods (fried rice) is important in recognizing safety problems relating to street foods. Fried rice is one of the most popular street food sold and consumed as it is both available and affordable. Despite its importance, little is known of the quality of these foods and their safety for human health. This research seeks to identify common food borne bacteria found in fried rice and determine their susceptibility pattern.

Materials and Methods

Sample collection

A total of six (6) samples were randomly selected from various foods vending site at boys' town market. The samples were collected using sterile universal containers. Samples were purchased along side other costomer and was immediately transferred under aseptic condition to the Laboratory at Adventist University of Wet Africa, for microbiological analysis within one hour of collection for analysis.

Sampling method

Sample of rice (3g) was dissolved in 100mls of water, homogenized and labeled A. One millilitre (1ml) of the suspension was removed with sterile pipette and transfer into 9ml of water blank and allow to mixed thoroughly and labeled B. This dilution was repeated three times, each time with 1ml transferred from previous suspension into 9ml water blank labeled sequentially as tubes C, D and E. These will result in serial dilutions of 0.1, 0.01 and 0.001, rice /ml.

Isolation of bacteria

Serial dilution was carried out in five sterile test tubes with 9ml of distilled water. One ml of the homogenized fried rice samples was introduced into the first tube containing distilled water and was allowed to mix properly. One millilitre (1ml) was withdrawn from the first test tube using a sterile pipette and was transferred into the second test tube. This process was repeated for the remaining test tubes. An aliquot of 0.1ml was withdrawn using a sterile pipette from test tubes 3 and 5 (0.001and 0.00001) and was inoculated into the sterile petri dishes containing already

prepared agar (nutrient agar, EMB agar and McConkey agar). The culture plates were incubated at 37°C for 24 hours. After incubation, colonies were picked up with wire loop and were sub-cultured into petri dishes containing freshly prepared nutrient agar to achieve pure isolate.

Identification of bacterial isolates:

After incubation, bacteria was identify based on the cultural, morphological and reaction to biochemical test (Cheesbrough, 2000; Cowan, and Steel, 1974; Cruikshank et al., 1975).

Determining the Susceptibility profile of the bacteria isolates

In testing for antimicrobial susceptibility, eight antibiotics from different classes (Siddiqui *et al*., 2018). Multidrug-resistant (MDR) organisms was chosen based on their resistance against three or more classes of antibiotics (Magiorakos et. al., 2011). Equally, the multiple antibiotic resistances (MAR) guide of the selected organism was determined as formerly described by (Krumperman, 1983).

RESULTS

In this study, a total of seven (7) bacteria (3- *Proteus*, 2 – *Citrobacter, 1- Pantoea* and *1- Serratia*) were obtained in this study from fried rice and the percentage occurrences of bacteria were 42.8%- *Proteus*, 28.6% – *Citrobacter, 14.3%- Pantoea* and *14.3% - Serratia* as seen in table 1.

Table1: Microscopic and Biochemical Identification of the isolates

SN	Isolates	MICROSCOPIC IDENT.		BIOCHEMICAL TESTS												PROBABLE ORGANISMS
		GRAM (+/-)	SHAPE	MT	IND	MR	VP	CT	SH	OX	CA	SUGAR TEST				
												G	L	M	S	
1	JPT 001	-	Rod	+	+	+	+	+	-	-	+	+a	+a	+a	+ag	*Proteus*
2	JPT 002	-	Rod	+	+	-	+	+	-	-	+	+a	+a	+a	+a	*Pantoea*

3	JPT 003	-	rod	+	-	+	+	+	-	-	+	+ a	+ a	+a	+a g	*Serratia*
4	JPT 004	-	Rod	+	+	+	+	+	-	-	+	+ a	+ a	+a	+a g	*Proteus*
5	JPT 005	-	Rod	+	-	+	-	+	-	-	+	+ a	+ a	+a	+a	*Citrobacter*
6	JPT 006	-	Rod	+	-	+	-	+	+	-	+	+ a	+ a	+a	+a	*Citrobacter*
7	JPT 007	-	Rod	+	+	+	+	+	-	-	+	+ a	+ a	+a	+a	*Proteus*

Key:
Gram's stained: (-) = Gram-negative Acids= +a, Acid & Gas and Negative fermentable sugar = (-) (no color changed or gas produced) CT = Citrate, OX = Oxidase, VP =Voges-Proskauer test, SH = Starch hydrolysis, CA = Catalyst, MT = Motility, MR = Methly Red,IND = Indole,G = Glucose,L = Lactose, M = Maltose,S= Sucrose

Table 2: Identified organism in fried rice pre vendor

Key: (-) Negative (+) Positive

S/N	Organism	Vendor 1	Vendor 2
1.	*Proteus*	+	-
2.	*Pantoea*	+	-
3.	*Serratia*	+	-
4.	*Proteus*	-	+
5.	*Citrobacter*	-	+
6.	*Citrobacter*	-	+

7.	*Proteus*	-	+

4.3 Antibiotics Sensitivity

A total of eight antibiotics were used in this study which include Ceftazidime (30µg) = CAZ, Cefuroxime (30 µg) = CRX, Gentamicin (10 µg) = GEN, Cefixime (6 µg) = CXM, Ofloxacin (5 µg) = OFL, Augmentin (30 µg) = AUG, Nitrofurantoin (300 µg) = NIT, Ciprofloxacin (5 µg) = CP. The result showed that most of the isolates were highly resistant to CRX, CXM, AUG, NIT and CPR as seen in Figure 1.

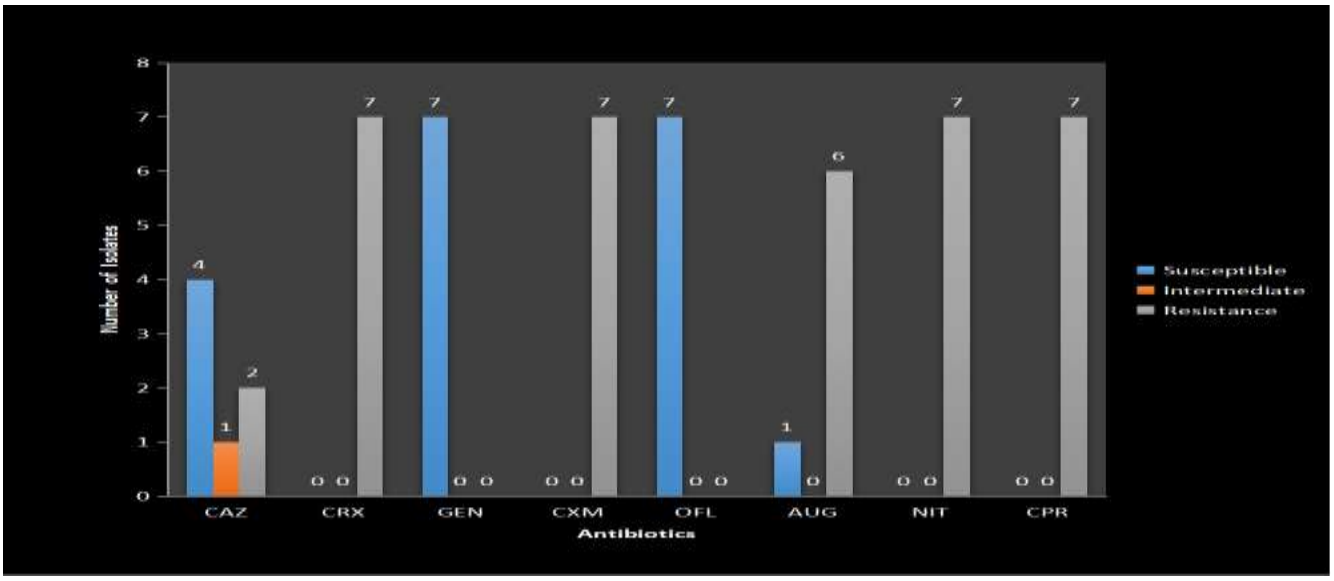

Figure: 1 Sensitivity Profile of the isolates

Key: Ceftazidime (30µg) = CAZ, Cefuroxime (30 µg) = CRX, Gentamicin (10 µg) = GEN, Cefixime (6 µg) = CXM, Ofloxacin (5 µg) = OFL, Augmentin (30 µg) = AUG, Nitrofurantoin (300 µg) = NIT, Ciprofloxacin (5 µg) = CP

Discussion

Microbial contamination is an indicator of the degree of safe handling of food which is a globally recognized vehicle for transmission of pathogens. All the food items examined in this study were contaminated in varying degrees and can be categorized as not satisfactory in terms of microbial quality. This agrees with reports of several investigators that street foods in some African and low income countries contained enteric pathogens of insignificant proportions (Beshiru et al ., 2020; Igbinosa et al., 2019). The presence of coliform bacteria in almost all food samples may be an indication of unhygienic handling of the food after cooking and or lack of good manufacturing practices which may account for post processing contamination recorded in this study. It is also an indication of post processing faecal contamination due to poor handling. Food handlers are normally directly responsible for contamination of foods with enteric pathogens in particular, during preparation, post-processing handling and cross-contamination also due to attitude of the food handler and vendors (Haileselassie et al., 2012). This highlights the epidemiological significance of the role of food handlers or vendors in Foodborne illnesses worldwide.

The results showed that *Proteus* was the most common coliform recovered in the Rice was Proteus which is a genus of Gram-negative Proteobacteria. Proteus bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, the mammalian intestine, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial. Proteus is an ecologically versatile bacterium, known to adapt to a variety of environmental conditions encountered in both animal hosts and the external environments. This attribute may, therefore, have been responsible for the presence of the organism in the ready-to-eat foods examined in this study. Other bacteria detected include *Citrobacter, Pantoea* and *Serratia* However, pathogens have been recovered from foods, or have been known to survive and grow in such foods. Reports from both developing and the developed nations suggest that the majority of food-borne gastrointestinal illnesses occur as a result of unhygienic handling and or the unsanitary environment during and after the food preparation. Humans are primarily exposed to gastrointestinal pathogens through direct or indirect contact with human and animal faecal wastes, or contamination of food (Olasupo et al., 2002). Evidence indicates that the incidence of diarrhoeal diseases can be reduced by preventing or controlling exposure to enteropathogens that are frequently present in foods (Madic et al., 2011).

In this study the result show that all the organisms show high resistance to the antibiotics used. Antibiotic resistance among the enteric bacteria recovered from cooked food revealed that microbes are becoming resistance to antibiotics that are commonly used to treat and cure infections both in human and veterinary medicine. Other reports have highlighted the existence of unacceptable high antibiotic residues in meat in Kenya and Nigeria (Muriuk et al., 2001; Dipeolu et al., 2002) indicating that the use of antimicrobials for animal husbandry in Africa is not a rare occurrence and may suggest misuse of antimicrobial agents. The high antibiotic resistance and multiple antibiotic resistances (MAR) observed among the bacteria recovered from food samples in the study area is, therefore, a cause for concern to public health. However, (Teuber et al.1999) reviewed the significant role of food and food-borne pathogens in food chain in the epidemiology of antibiotic resistant bacteria, thus highlighting the need for urgent attention, particularly in low resources countries that may not possess the means to curtail any major outbreak by such MAR organisms. The occurrence of antibiotic-resistant commensals in ready-to-eat retailed foods has been reported (Van et al.2997). In their study, reported that more than 90% of foods sampled were contaminated with E. coli and 83.3% of the E. coli was resistant to at least one antibiotic. Furthermore, the role of commensals, especially food-borne microbes in transmitting antibiotic resistance genes through horizontal transfer has been highlighted. The present study emphasizes the importance of surveillance of bacterial isolates throughout the food production continuum to detect emerging antimicrobial resistance phenotypes in Liberia. These may include but not limited to an increased number of hospitalization, increased risk of invasive infections, and failures in medical treatment, increased health costs and mortality.

Food safety in the food market is one of the key areas of focus in public health because it affects people of every age, race, gender, and income level around the world. The local and international food marketing continues to have significant impacts on food safety and the health of the public. Food supply chains cross multiple national borders which increase the internationalization of health risks. Overall, this study suggests that food safety-related public health risks are more common in developing countries than in developed countries. This can be justified that foods get easily contaminated with microbes due to poor hygiene and sanitation in developing countries (Baluka et al., 2015). In recent years, several global and national public health organizations have

highlighted the growing number of multidrug-resistant microbes as a major public health priority. Food plays an important role in the transmission of Foodborne pathogens.

Transmission of microorganisms between food and humans occurs during the handling of raw materials as well as cross- and re-contamination between different food products at production, distribution and household levels. Approximately 15% of emerging infectious disease events have been associated with Foodborne transmission (Parmley et al., 2012). This is of concern, given that the interconnectedness of global food systems is resulting in increased antimicrobial resistant Foodborne disease transmission. Antimicrobial resistance is now widely acknowledged as a major global public health challenge (FAO, 2007) Food contamination with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance. In low- and middle-income countries, antimicrobial resistance remains largely unaddressed. The World Health Organization states that "large gaps in knowledge exist about the status of antibiotic resistance surveillance capacities worldwide, particularly in resource-limited settings (WHO, 2015). Inappropriate antibiotic prescriptions, over-the-counter availability, poor patient adherence to prescribed medications, use of substandard medications, and self-medication with previously unused antibiotics all increase the development of antimicrobial resistance. The use and misuse of antibiotics, resistance can develop in bacteria in human beings and animals. Hence, infections that normally respond to antibiotic treatment can become difficult and sometimes impossible to cure. The resulting treatment failures lead to increased disease cases and deaths, a growing challenge to develop new antibiotics and consequently higher costs to society.

Conclusion:

The occurrence of these bacterial isolates in the foods could result in a public health threat to consumers as all these bacterial pathogens have been associated with diarrheal illness and other Foodborne infection. The identification of these bacteria in the foods samples could be attributed to poor personal hygiene, noncompliance to hazard analysis and critical control point's scheme. To minimize this unwholesome trend of ready-to-eat food contamination, it is important for appropriate agencies in food safety and public health to organize training on hygiene and food safety for food vendors.

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