Original Research Article

ISOLATION IDENTIFICATION AND ANTIBIOGRAM OF *Pseudomonas aeruginosa, Klebsiella pneumonia* AND *Escherichia coli* ISOLATES FROM READY TO EAT FOOD SAMPLES IN ULI CAMPUS.

ABSTRACT

Food-borne disease outbreak have imposed substantial burden on health care systems and have markedly reduced the economic productivity of the country. Ready to eat food are well known source for bacteriological contamination. They contain pathogens responsible for health hazard like food poisoning and diarrheal disease. This study aims to evaluate the presence of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* in of fried rice, jollof rice, turkey and chicken as well as antibiogram profile of the isolates incriminated. A comparative study of the food samples was carried out using standard procedures for isolation and identification of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli.* The antibiotics sensitivity disc was used to determine the antibiogram profile of the isolates. The prevalence of *E. coli*, *K. pneumonia* and *P. aeruginosa* and in food samples was found to be 68.45%, 20.24% and 11.31% respectively. *E. coli* and *K. pneumonia* was susceptible to Tarivid 10µg, Reflacine 10µg, Ciprofloxacin 10µg and Streptomycin 30µg while *P. aeruginosa* was resistant to the antibiotics used. These data revealed the presence of *E. coli*, *K. pneumonia* and *P. aeruginosa* from different ready to eat foods as well different reactions to certain antibiotic tested.

KEYWORDS: Food safety, Antibiogram, Food quality, Hygiene Standards, Food microbiology.

ABBREVIATIONS

- RTE: Ready to Eat
- CLSI: Clinical Laboratory Standard Institute
- COOU: Chukwuemeka Odimegwu Ojukwu University

INTRODUCTION

The definition of food can be said to entail any substance that is consumed in order to give both essential and non essential nutrients to the consumer. They can be plant-based, animal-based, fungi and can contain nutrients like Carbohydrates, fats proteins, vitamins, minerals. The assimilated food is being broken down and the nutrients absorbed by the organism's cells to provide energy, stimulate growth and other beneficial and non beneficial attributes [1].

“Ready-to-eat foods are foods that are produced for direct consumption without the necessitating cooking or other processing activities which aimed at reducing or eradicating the microbial load to improve its acceptability. In as much as Ready-to-eat (RTE) foods have some advantages, there are they have health implications such as food-borne illnesses. Food-borne illnesses (food poisoning) are diseases that are resultants from ingesting contaminated foods" [2]. New South Wales Food Authority (NSWFA) has defined ready-to-eat foods originally as foods that are consumed in the same state in which they are sold and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling. These foods can cause harm due to the fact that they support survival and proliferation of pathogens when not properly handled. They enhance the transmission of food borne pathogens in human population [3]. Some authors have described them as a vehicle for the transmission of microbial diseases, some of them which are caused *E. coli* and other medically important bacteria [4-6]

US Department of Health and Human Services (USDHHS) defined food-borne illnesses or food poisoning as diseases that are acquired as a result of eating contaminated food [2]. Food poisoning can be a concomitant of eating contaminated food with considerable amount of viable pathogens. Food-borne illnesses are part of disease conditions which leads to morbidity and mortality all over the world [7]. Bacterial pathogens have been implicated in a number of food-borne diseases recently, and some of these microbes have proven to be resistant to some available antimicrobial agents [4,5,8]. Improper handling of foods plays a vital role in transmission of food-borne pathogens such as *Escherichia coli* and *Klebsiella pneumoniae* to unsuspecting individuals who eat them. In addition, infections can also occur from toxin production by the organisms. The consumption of such foods can be seen at picnics, restaurants or fast food joint and as a result there is need to set up food-traceability systems so as to improve the quality of food processing events and ensure that there is safe food for the final consumers [9].

Ingestion of foods contaminated with considerable number of viable pathogens can lead to food poisoning. Improper handling of food materials can lead to transmission of food-borne pathogen which includes *Escherichia coli* and *Klebsiella pneumoniae* to consumers [10].

“The prevalence of a good number of food-borne diseases in recent times have been said to be caused by certain bacterial pathogens of which some of them have antimicrobial resistance on antimicrobial agents. Such bacteria includes *Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella, Escherichia coli, Clostridium botulinum,* and *Clostridium perfringens”* [11].

The study of the antibiogram profiles of certain organisms is an important tool for monitoring the antibiotic resistance and enlightens on the resistant pattern of the organisms over certain period. It aids in the selection of antibiotics to adequately treat infections. Antibiotics has been utilized so as to curb the morbidity, mortality and averse effect if bacterial infections on the economy. The use of antimicrobials in food production is geared into prevention of illnesses; improve health and the improvement of Livestock [12]. When antibiotics are being administered at low

doses, for a long period of time in feeding of animals, they can result to selection and spread of antibiotic resistance to other microbes [13]. Consumption of certain plant-based foods especially salad and other RTE street foods containing multidrug resistant isolates have become a serious concern. Some authors have reported the presence of multidrug resistant and extended spectrum beta lactamase producing E.coli from different sources which includes raw meat, vegetable salad, egg surface, unpasteurized milk, raw fish, and water, indicating major public health concerns [14,15,]. Some foods which are exposed or are in contact with sewage pollution can serve as vehicles for transmitting pathogens to humans [16].

Certain factors have led to emergence and more appreciation of RTE foods. Such factors includes, rapid increase in population, changes to certain modern lifestyles, longer working hours, wider integration of women into the labour force, changes in cooking and eating habits. Some of the inhabitants of urban areas that have less time to make home-made meals due to the their busy schedule hence uses RTE foods as major components of their daily diets and that has also led to the increase rate of consumption of RTE foods [7]. Considering also the fact that these foods are shelf-stable, affordable, colourful, easily accessible to the consumers and saves time, people gear towards it consumption [17,18]. These foods if not properly handled or care taken in its preparation, it could lead to full blown food-borne disease outbreak [19]. The aim of this study is isolate, identify and determine the antibiogram profile *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* isolates present in RTE samples in Uli campus of Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.

MATERIALS AND METHODS

2.1 Sample Collection

"A bacteriological survey was conducted in different types of ready to eat food in Chukwuemeka Odumegwu Ojukwu University Uli campus. Uli is a town in Anambra state of Nigeria with land coverage of 256km^2. It is located at 5°47'N 6°52'E of Nigeria. Five fresh warm samples of the each of the food samples which includes fried rice, jollof rice, salad and fried chicken was randomly collected from 4 different food vendors within the school campus. The samples were transported to the Microbiology Laboratory of COOU, Uli where they were analyzed following standard microbiology techniques" [20].

2.2 Culturing of the sample

A clean sterile covered plate was used to dish the food and each of the food samples was macerated using a sterile marble mortar. Serial dilutions of the food substances was carried out using a sterile distilled water before plating out in the appropriate growth media.

The media used includes Eosine methylene blue, Chromocult and Cetrimide which was prepared according to the manufacturer's instructions. The media was inoculated with the dilutions of the food substances using the pour plate method and incubated at 37°c for 24hours. All growth media were procured from Oxoid (Oxoid, UK).

"Suspected colonies of *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* species from the selective media was subcultured into nutrient agar slants for storing from which they were collected for the biochemical tests" [20].

2.3. Bacterial identification and characterization

The cultures were characterized to the genus level of the various bacterial groups using biochemical tests. The biochemical tests performed includes: Methyl red test, Catalase, Citrate, Oxidase and Sugar fermentation test [20]. Gram-staining was conducted following standard procedures in order to observe the colors and shapes of the cells.

2.4 Antibiotic Susceptibility Testing of Isolates

Antimicrobial susceptibility test was performed on Mueller-Hinton (Oxoid, UK) agar plates by the Kirby-Bauer disk diffusion method as per the Clinical Laboratory Standard Institute (CLSI) criteria [21]. The tested antibiotics included Tarivid (OFX) 10µg, Reflacine (PEF) 10µg, Ciproflox (CPX) 10µg, Streptomycin(S) 30µg. The bacteria isolates were diluted in saline to obtain turbidity equivalent to 0.5 McFarland standards. Aliquots were seeded by swabbing on Mueller-Hinton agar plates, with subsequent application of the antibiotic disks. The plates were incubated at 37˚C and interpreted using meter rule as per CLSI criteria [21].

RESULTS

A total of 168 bacterial pathogens were obtained from 180 culture positive samples from ready-to- eat foods sold around COOU, Uli campus. Overall *E. coli* (68.45%) *(*a uropathogen that indicates feacal contamination) was the was the most prevalent bacteria isolated followed by *K. pneumonia*(20.24%) and then *P. aeruginosa* (11.31%).

The result on Table 1 showed the distribution of isolated bacterial isolates present in the RTE foods. It showed a total number of 34 isolates of *Klebsiella pneumonia*, 115 isolates of *Escherichia coli* and 19 isolates of Pseudomonas *aeruginosa*. The statistical analysis of the food samples and isolates presented that there is no significant difference in the number of organisms found in the different food samples. The percentage distribution of the isolates was represented in the pie chart in Figure 1. The result shows that the percentage distribution of *Klebsiella pneumonia* is 20.24%, *Escherichia coli* is 68.45% and *Pseudomonas aeruginosa* is 11.31%. This result shows that E.coli is the most predominant isolate in the food samples evaluated.

Table 1: Distribution of the isolated bacterial pathogens from the food samples

Food samples	*Escherichia coli* (n)	*K. pneumoniae* (n)	*P. aeruginosa* (n)	Total	Average
Fried rice (n=5)	30	10	4	44	14.66667
Jollof rice (n=5)	25	10	7	42	14
Salad (n=5)	20	5	3	28	9.333333
Fried chicken (n=5)	40	9	5	34	11.33333
Total	115	34	19	168	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	54.66666667	3	18.22222	0.171503	0.912671	4.066181
Within Groups	850	8	106.25			
Total	904.6666667	11				

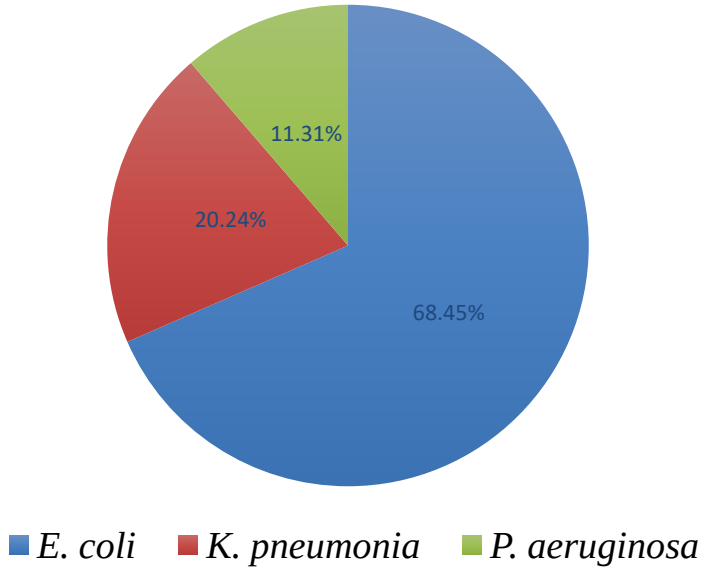

Figure 1. Pie Chart showing the percentage distribution of the Bacterial isolate

The biochemical identification of the isolates are represented in Table 2, the isolated bacteria were identified using Gram reaction, colony characteristics and biochemical tests.

Table 2: **Identification of the bacterial isolates**

Features	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***
Colony characteristics	Cocci	Rod	Cocci
Gram Staining	-ve	-ve	-ve
Colony colour on the media	Mettalic green	Pink mucoid colonies	Bright green
Catalase test	+ve	+ve	+ve
Oxidase test	-ve	-ve	+ve
Citrate test	-ve	-ve	+ve
Methyl red test	+ve	-ve	-ve
Fructose	-ve	-ve	-ve
Glucose	+ve	+ve	-ve
Lactose	+ve	+ve	-ve
Maltose	-ve	+ve	-ve
Mannitol	-ve	+ve	+ve
Sucrose	+ve	+ve	+ve

Key: +ve- Positive; -ve- Negative.

The result on Table 3 showed the antibiogram profile of the isolates from the food sample as per the Clinical Laboratory Standard Institute (CLIS) criteria. The tested antibiotics were Tarivid (OFX) 10µg, Reflacine (PEF) 10µg, Ciproflox (CPX) 10µg, Streptomycin(S) 30µg. *E. coli* and *K. pneumonia* was susceptible to Tarivid 10 µg, Reflacine 10µg, Ciproflox 10µg and Sterptomycin 30µg while *P. aeruginosa* was resistant to the antibiotics used.

Table 3: **Antibiotics susceptibility profile of the bacterial isolates from the food samples**

Isolates	Zones of inhibition (mm)				
	OFX	PEX	CPX	S	% Susceptibility
Escherichia coli	24	22	28	17	100
K. pneumoniae	15	25	33	18	100
P. aeruginosa	0	0	0	0	0

OFX- Tarivid; PEX- Reflacine; CPX- Ciprofloxacin; S-Sterptomycin

1. DISCUSSION

There is a lot of food vendors located in different Nigerian campuses which students tend to patronise for the purchase of ready to eat foods [10]. Some of the reasons why certain students patronize these food vendors could range from time constraint or inability to make their food themselves. As a result, they are at high risk of exposure to food-borne diseases due to poor handling and poor preparation of these foods by vendors, which allows pathogenic microorganisms to thrive in them and cause infection upon consumption.

A total of 168 bacterial pathogens were obtained from 180 culture positive samples from ready-to- eat foods sold around COOU, Uli campus. Overall *E. coli* (68.45%) *(*a uropathogen that indicates feacal contamination) was the was the most prevalent bacteria isolated followed by *K. pneumonia* (20.24%) and then *P. aeruginosa* (11.31%).

The prevalence of *E.coli* in this study could be said to be due to contamination from the different human part into the food. In addition, the presence of such Gram-negative organisms could mean potential faecal contamination of the food. Isolation of those organisms from the samples is a pointer that these products were subjected to unhygienic practices such as too much personnel handling, use of poor-quality water during processing and undue exposure during retailing. *E. coli* can survive in areas with low water activity hence increasing presence in food samples generally considered as being microbiologically safe [10]. The presence of *K. pneumoniae* could be as a result of lack of personal hygiene, transmission of aerosols through coughing or sneezing by customers or vendors. Presence of *P. aeruginosa* in these could be as a result of lack of personal hygiene of vendors and improper cleaning of cooking utensils which can give rise to formation of food layers that allows biofilm formation.

This work is also in line with the work of Oluyege *et al* [22] who also reported the presence of *Escherichia coli* and *K.pneumoniae* in RTE foods sold in a University campus in south western Nigeria. The result of this work is in line with the work of Nataha *et al* [23] who reported the presence of *E.coli* and *Klebsiella pneumonia* in taco dressing in Mexico.

Ciprofloxacin is one of the broad-spectrum antibiotic which means it is effective for both gram positive and gram negative organisms. It is more sensitive to Gram negative than Gram-positive bacteria. The *E.coli* isolated in this study was susceptible to the ciprofloxacin testes but this was not the case of because it showed resistance to the ciprofloxacin as well as all the antibiotics tested. This report is in line with the antibiotics susceptibility assay of *Escherichia coli, S. aureus and Salmonella typhi* against ciprofloxacin reported by Ali *et al*.[24]. The *E.coli* isolated was sensitive to all the antibiotics tested including Ofloxacin (Tarivid is the brand name for Ofloxacin), this result is not in agreement with the report of Iroha *et al* [10] who recorded that the *E. coli* isolate from their work was resistant to Ofloxacin. The presence of *E.coli* in this work is also in line with the work of Ema *et al*., [7]. who also isolate *E.coli* in RTE foods in Bangladesh.

Following the studies that have been carried out with on and outside Nigeria, it can be seen that *E.coli* and other enteric and no enteric pathogens like *K.pneumoniae* and *P.aeruginosa* have been playing a vital role in global cases of food poisoning [4,6,25]. This information could be said to be somewhat true considering that the mentioned organisms were also reported in this study in Table 1. The improper handling of these RTE foods and using of contaminated materials for the preparation of these foods could be a contributing factor to the worrisome frequency of these food pathogens in food.

CONCLUSION

This study has demonstrated that some of the most popular types of ready-to-eat foods that are sold in canteens and cafeteria of Uli Campus are contaminated with *Escherichia coli, K. pneumonia* and *P. aeruginosa* hence do not meet the required quality and safety levels. These organisms present in food samples have the potentials of causing gastroenteritis. Improper handling of RTE could lead to cross contamination as *E. coli* demonstrate faecal contamination. Hence there is need to practice proper hygiene while handling RTE foods to prevent spread of pathogens and dissemination of diseases. There is need for sensitization and enlightening food vendors on the need to practise safe hygiene while handling these RTE foods.

COMPETING INTERESTS:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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