Original Research Article

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

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ABSTRACT

Food-borne disease outbreak have imposed substantial burden on health care systems and have markedly reduced the economic productivity of a country. Ready to eat food are well known source for bacteriological contamination with pathogen responsible for health hazard like food poisoning and diarrheal disease and they play significant role in transfer of antibiotic resistance. This study aims to evaluate bacteriological status of fried rice, jollof rice, turkey and chicken and the Antibiogram. 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* as potential reservoir of human infection and transmitters of antimicrobial resistance. 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* showed good level of susceptibility to Tarivid 10mcg, Reflacine 10mcg, Ciproflox 10mcg and Streptomycin 30mcg. While *K. pneumonia* had fairly good susceptibility to Ciproflox 10mcg, Streptomycin 30mcg and Gentamycin 10mcg and *P. aeruginosa* was resistant to the antibiotics used. These data revealed also that the *E. coli*, *K. pneumonia* and *P. aeruginosa* isolates recovered from the food samples were resistant to multiple antimicrobials, which can be transmitted to humans through food products.

KEYWORDS: Ready to Eat (RTE) foods, Antibiogram, Hygiene Standards, Microbiological Quality.

ABBREVIATIONS

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

1. INTRODUCTION

Bacteria are microscopic, single-celled organisms that exist in their millions, in every environment, both inside and outside other organisms. Some bacteria are harmful, but most serve a useful purpose. They support many forms of life, both plant and animal, and they are used in

industrial and medicinal processes. The shapes of important bacteria are classified into-cocci or spherical cells, bacilli or cylindrical or rod-shaped cells, and spiral or curved forms. Different strains of bacteria are also used in production of various food and dairy products [1].

Food is any substance consumed to provide nutritional support for an organism. Food is usually of plant, animal or fungal origin, and contains essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells to provide energy, maintain life, or stimulate growth [2].

Ready-to-eat food is food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organism of concern. Ready-to-eat (RTE) foods, despite having many advantages, are associated with the occurrence of foodborne illness cases and outbreaks raising concerns about their safety. Food-borne illnesses (which are commonly referred to as food poisoning) are diseases that results from eating contaminated food [3].

Food poisoning can ensue after eating food contaminated by considerable number of viable pathogens, and this commonly occurs after eating at picnics, restaurants or fast food joint. Poor handling of these foods plays critical role in the onward transmission of food-borne pathogens including *Escherichia coli* and *Klebsiella pneumoniae* to unsuspecting patronisers who eat them [4].

Bacterial pathogens have been implicated in a handful of food-borne diseases in recent times, and these microbes are resistant to some available antimicrobial agents. However, bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella, Escherichia coli, Clostridium botulinum*, and *Clostridium perfringens* can survive and grow under the acid conditions in these acidic RTE foods, posing a health risk [5].

Antibiogram study is an important tool for antibiotic resistance monitoring and provides a review on the resistance pattern over a period. It also aids in evidence-based selection of antibiotics for empirical treatment of infections in an area. The aim of this study is to look at the antibiogram of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* isolates recovered from ready to eat food samples in Uli campus of Chukwuemeka Odimegwu Ojukwu University, Anambra State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

A bacteriological survey was conducted in different types of ready to eat food including fried rice, jollof rice, salad and fried chicken which were aseptically and randomly collected from 4 different food vendors within and around the School campus. The samples were transported to the Microbiology Laboratory of COOU, Uli where they were analyzed following standard microbiology techniques [6]. Two main assessments were carried out; isolation and identification of *Escherichia coli, K. pneumoniae and P. aeruginosa* to determine their level of bacterial contamination and safety for human consumption and antibiotic susceptibility testing on the food isolates.

2.2 Culturing of the sample

A clean sterile covered plate was used to dish the food and each of the food sample was macerated using a sterile marble mortar.

Then 1g of each food sample was homogenized in sterile water and the volume of the homogenate was made up to 10ml to obtain a 1: 10 suspension. 1ml of the suspension was pipetted into the petri dishes for serial dilution and 1ml of 10^{-3} was pipetted into 5 different petri dish each for the four samples.

The media which are Eosine methylene blue, Chromocult and Cetrimide was prepared according to the manufacturer's instructions. Briefly each plate was carefully labelled on top and shaking of these plates were done as soon as the agar was poured, so as to have the microorganisms separated during growth. The media was allowed to set on a flat top bench after which plates were incubated at 37°c for 24hours.

2.3 Sub-culturing

The colonies were sub-cultured in fresh EMB agar, Chromocult agar and cetrimide agar plates. The plates were incubated at 37°c for 24 hours. Suspected colonies of *E. coli, klebsiella* and *pseudomonas* species were transferred to nutrient agar slants for storing from which they were subjected to gram staining, fermentation test, catalase, oxidase, citrate and methyl-red test for proper identification.

2.4 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. Gram-staining was conducted following standard procedures in order to observe bacterial cell shapes (spherical, rod, spiral, etc.) and arrangements (single, pair, chain, clusters, tetrads, etc.).

2.3 Sugar Fermentation Test

The various sugars such as Glucose, Fructose, Galactose, Maltose, Mannitol, Lactose and Sucrose were prepared according to manufacturers guide and allowed to warm to room temperature. Preparation of bromothymol blue as indicator – I dissolved 20ml of ethanol in 5g of bromothymol blue and added drop of sodium hydroxide until it changes to blue colour. Then I inoculated the bromothymol blue indicator with the carbohydrate with isolated colonies. I incubated aerobically at 35-37°c for 3-5 days and observed daily for development of yellow colour in the medium.

2.4 Antibiotic Susceptibility Testing of Isolates

Antimicrobial susceptibility test was performed on Mueller-Hinton (MH) agar, plates by the Kirby-Bauer disk diffusion method as per the Clinical Laboratory Standard Institute (CLSI) criteria (CLSI, 2010). The tested antibiotics included Tarivid 10mcg, Reflacine 10mcg, Ciproflox 10mcg, Augmentin 30mcg, Gentamycin 10mcg, Streptomycin 30mcg, Ceporex 10mcg, Nalidixic acid 30mcg, Septrin 30mcg, Amplicin 30mcg. The bacteria isolates were diluted in saline to obtain turbidity equivalent to 0.05 McFarland standard. 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 [7].

3. RESULTS

The sample of ready to eat food collected from the food vendors around Uli campus were tested to isolate *Escherichia coli, K. pneumoniae and P. aeruginosa* to determine the antibiogram of those bacteria isolates.

Table 1 and Figure 1 showed the distribution of isolated bacterial and the percentage distribution respectivelyas, 34 *Klebsiella pneumonia* (20.24%), 115 *Escherichia coli* (68.45%) and 19 *Pseudomonas aeruginosa* (11.31%). As shown in table 2, the isolated bacteria were identified using Gram reaction, colony characteristics and biochemical test.

Table 3 showed the results of antibiogram of the isolates from the food sample using the Kirby-Bauer disk diffusion method as per the Clinical Laboratory Standard Institute (CLIS) criteria. The tested antibiotics were Tarivid (OFX) 10mcg, Reflacine (PEF) 10mcg, Ciproflox (CPX) 10mcg, Augmentin (AU) 30mcg, Gentamycin (CN) 10mcg, Streptomycin(S) 30mcg, Ceporex (CEP) 10mcg, Nalidixic acid (NA) 30mcg, Septrin (SXT) 30mcg, Amplicin (PN) 30mcg. *E. coli* showed good level of susceptibility to Tarivid 10mcg, Reflacine 10mcg, Ciproflox 10mcg and Streptomycin 30mcg. While *K. pneumonia* had fairly good susceptibility to Ciproflox 10mcg, Streptomycin 30mcg and Gentamycin 10mcg and *P. aeruginosa* was resistant to the antibiotics used.

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

Food samples	***Escherichia coli***	***K. pneumoniae***	***P. aeruginosa***
Fried rice (n=5)	30	10	4
Jollof rice (n=5)	25	10	7
Salad (n=5)	20	5	3
Fried chicken (n=5)	40	9	5
Total	115	34	19

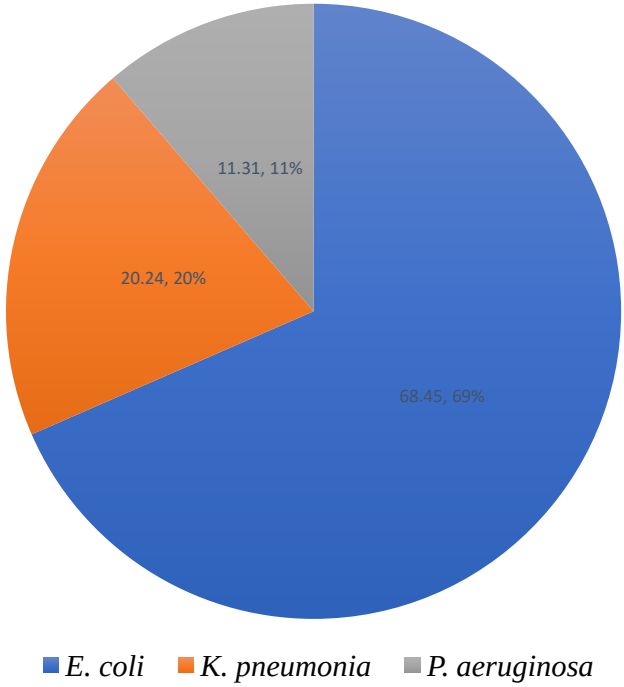

Figure 1 : Pie Chart showing the percentage distribution of the Bacterial isolate

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

Features	***Escherichia coli***	***K. pneumoniae***	***P. aeruginosa***
Colony characteristics	Cocci	Rod	Cocci
Gram character	-ve	-ve	-ve
Microscopic feature	Mettalic red	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

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

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

4. DISCUSSION

The main objective of the present study was to isolate, identify and determining the Antibiogram of *Escherichia coli*, K. *pneumoniae* and *P. aeruginosa* from ready to eat food gotten from food vendors around Chukwuemeka Odimegwu Ojukwu University (COOU), Uli campus, Anambra state, Nigeria.

A handful of Nigerian students and other individuals depend mainly on food vendors, fast food centres and nearby restaurants that sell a variety of ready-to-eat foods for their daily meal. A number of reasons emerged from this rising development, but most importantly they patronize these fast food centres for want of time 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 observed high occurrence of *E. Coli* in this study could be attributed to its wide range of habitats including human body parts, which may be a source of contamination for the food. In addition, the presence of such Gram-negative organisms could mean potential fecal contamination of the food. Isolation of those organisms from the samples is a pointer that these products were subjected to unhygienic practices, too much personnel handling, use of poor-quality water during processing and undue exposure during retailing. Since *E. Coli* has the ability

to tolerate low water activity, this could also have contributed in their survival in exposed food samples generally considered as being microbiologically safe [4]. Contamination with *K. pneumoniae* might be due to poor personal hygiene, deposition of aerosols generated by coughing or sneezing by customers or vendors. Occurrence *of P. aeruginosa* in these foods might be attributed to inadequate personal hygiene of vendors and improper cleaning of utensils leading to food layer deposition that favors the growth of biofilms. According to WHO, there is a 25% diarrhoea in food borne illness caused by food infected with *E. coli*.

A study by Bhaskar *et al* [9] in Mangalore demonstrated 93% bacterial contamination out of 60 street food samples tested. In a Mexican study [10], a total of 103 taco dressings were sampled for *E. coli* and *Salmonella* sp. The study revealed 44 samples (43%) contained *E. coli* and 5 samples (5%) *klebsiella*. Feglo *et al.* [11] reported 100% contamination of various food samples collected from Kumasi, Ghana.

Among these foods, Salad showed highest bacterial contamination (28.57%) followed by Fried rice (26.19%). These foods were on high demand and hence prepared much earlier to consumption and kept uncovered exposed to dust and air pollution on roadsides. Least contamination was shown by Fried chicken (20.24%) probably due to their preparation shortly before consumption and repeated reheating as customers prefer to buy them hot.

Ciprofloxacin is regarded as a broad-spectrum antibiotic. It is more sensitive to Gram negative than Gram-positive bacteria. This study shows that all the *E. coli* isolates were sensitive to ciprofloxacin. However, *P. aeruginosa* isolates were resistant to ciprofloxacin. Antibiotics susceptibility assay of *Escherichia coli, S. aureus and Salmonella typhi* against ciprofloxacin reported by Ali *et al*.[12] shows some level of similarity with the findings from this study. A related study by Oluyege *et al*. [13] which involved antibiotic resistance profile of bacterial isolates from ready-to-eat indigenous foods such as pounded yam reported that *E. coli* which represent 2 (8.70%) of the bacterial isolates showed resistance to nalidixic acid; all the *K. pneumonia* isolates were resistant to gentamicin; 4 (40%) were resistant to nalidixic acid and 10 (100%) were resistance to Augmentin is similar to the results from this study.

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, and do not meet the required quality and safety levels. Some of the bacteria isolated especially *Escherichia coli* and *K. pneumonia* that are isolated in almost each and every collection of the food sample are potential enteric pathogens and are known to cause gastroenteritis. This clearly shows poor handling and management leading to cross contamination as *E. coli* demonstrate feacal contamination. This pose a health threat to the patron and efforts to reduce level of contamination in this canteens and

cafeterias are highly recommended as not only student from COOU Uli campus rely on the food, but also some individuals from the community as most dwellers are workers.

COMPETING INTERESTS DISCLAIMER:

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Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Yvette Brazier (2019) What are bacteria and what do they do? Medical News Today. https://www.medicalnewstoday.com/articles/157973 Accessed on 10th January, 2022.

2. Britannica (2022). Food. https://www.britannica.com/search?query=food Accessed on 10th January, 2022.

3. U.S Department of Health and Human Services (2013). Report of the FDA Retail Food Program Database of Foodborne Illness Risk Factors.

4. Iroha Ifeanyichukwu, Afiukwa Ngozi, Nwakaeze Emmanuel, Ejikeugwu Chika, Oji Anthonia and ILang Donathus (2014). Antibiogram of food-borne pathogens isolated from ready-to-eat foods and Zobo Drinks Sold Within and Around PRESCO Campus of Ebonyi State University (EBSU), Abakaliki, Ebonyi State, Nigeria. *Journal of Toxicology and Environmental Health Sciences*. Vol. 6(1), pp. 1-4

5. Adekemi, O., Ayokunle, C. D. andEyitayo, O. (2009). Antibiotic resistance profile of bacterial isolates from food sold on a University campus in south western Nigeria. *African Journal of Biotechnology* 8(21):5883-5887

6. Cheesbrough M (2000). District Laboratory Practice in Tropical Countries. Part II. 2nd edition. Cambridge University Press, UK. pp. 178-187.

7. Clinical Laboratory Standard Institute, CLSI (2010). Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standards CLSI M100-S20, Wayne, PA. USA

8. World Health Organization, Geneva. World Health Organization (WHO) (2008). *Microbiological risk assessment series.* Microbiological hazards in fresh fruits and vegetables.

9. Bhaskar, J., Usman, M., Smitha, S. and Bhat, G.K. (2004). Bacteriological profile of street foods in Mangalore. *Indian Journal of Medical Microbiology*22: 197.

10. Natascha, C., Julie B.,Ellen, L. and Inge, Van D.(2014). Co-occurrence of free-living protozoa and foodborne pathogens on dishcloths: Implications for food safety. *International Journal of Food Microbiology* 191:89–96

11. Feglo, P. and Sakyi, K. (2012). Bacterial contamination of street vending food in Kumasi, Ghana. *Journal of Medical and biomedical sciences* 1(1): 25-40.

12. Ali, S.Q. , Ale, Z., Baquar, S.N. and Shahjahan, S. (2010). Resistance Pattern of Ciprofloxacin Against Different Pathogens. Oman Medical Journal 25(4): 294-298.

13. Oluyege, A., Ayokunle, C.D., Ojo, A.M. and Eyitayo, O. (2009). Antibiotic resistance profile of bacterial isolates from food sold on a University campus in south western Nigeria. African Journal of Biotechnology 8(21): 5883-5887.

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