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ANTIBIOTIC ACTIVITIES OF*PSEUDOMONAS AERUGINOSA, KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES RECOVERED FROM FOOD SAMPLES IN ULI CAMPUS

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ABSTRACT

Food poisoning (food-borne disease) involvesinfections that occurs after consuming food contaminated by adequate numbers of viable pathogens and their toxins. The main aim of this study is to evaluate bacteriological pathogens present inmeat pie, egg roll, moi-moi, okpa, and zobo drinks and their Antibiogram of the food isolate. A comparative study of the food samples was carried out using standard procedures for isolation and identification of *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli* as potential reservoir of human infection and sources of antimicrobial resistance.The prevalence of *E. coli*, *K. Pneumonia* and *P. Aeruginosa*and in food samples was found to be 58.82%, 29.41% and 11.76% 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 that the *E. coli*, *K. Pneumonia* and *P. aeruginosa* isolates gotten from the food samples were resistant to various antimicrobials, which can be transmitted to humans through food products.

INTRODUCTION

Bacteria are minute microorganisms, single-celled that occur in masses, in all surroundings, both internally and externallyof other microorganisms. A few bacteria are destructive, but most of them are useful. The forms of bacteria are classified into-cocci or spherical cells, bacilli or cylindrical or rod-shaped cells, and spiral or curved forms. (Oxford,2021).

Any substance consumed to offer nutritive sustenance for an organism is known as Food. Food is typically of plant, animal or fungal origin, and comprises essential nutrients, for example carbohydrates, fats, proteins, vitamins, or minerals. The food is consumed by an organism and digested by the cells of the organisms to make available energy, sustain life, or stimulate development. (Britannica, 2017)

Food is a substance proposed by the manufacturer for direct human consumption without the need for heating or other processing which are used to inhibit or decrease to a tolerable level of microorganism of concern. Foods, regardless of its advantages, are linked with the incidence of foodborne disease cases raising concerns about their safety. Food-borne diseasesalso known as food poisoning) are diseases that occurs from eating infected food (USDHHS, 2013).

Bacterial pathogens have been implicated in a few of food-borne diseases in current times, and these pathogens are resistant to some obtainable antibiotics. Nevertheless, bacteria for instance *Pseudomonas aeruginosa, klebsiella pneumonia, Salmonella, Escherichia coli, Clostridium botulinum,* and *Clostridium perfringens* can live and grow under the acidic environments in these acidic foods, posing a health risk (Chavatte*et al*., 2014).

The study of antibiogram is a vital tool for antibiotic resistance monitoring and offers a review on the pattern of resistance over a period.

Materials and methods

Sample Collection

A bacteriological survey was conducted in different types of ready to eat food including moi-moi, okpa, egg roll, meat pie and zobo drinks 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 anlysis was carried out using standard microbiology techniques. 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.

Method

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 agar and Cetrimide agar to be used was prepared according to the manufacturer's instructions. Briefly each plate was carefully labeled 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.

Sub-culturing

The colonies were sub-cultured in fresh EMB, 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.

Biochemical analysis of various isolates obtained in the cultures

Bacterial identification and characterization

The cultures were characterized to the genus level and/or various bacterial groups using biochemical tests. The biochemical tests performed includes: Methyl red test, Catalase and Oxidase. 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.).

Sugar fermentation test

The various sugars such as Glucose, Fructose, Galactose, Maltose, Mannitol, Lactose and Sucrose were prepared according to manufacturer's guide. Sugar fermentation tests an organism's ability to ferment the sugar glucose. The development of yellow color in the medium indicates for positive.

Antibiogram

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 for 24hours and interpreted using meter rule as per CLSI criteria.

RESULT

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 shows the total number of isolated bacterial to be, 60 *Klebsiella pneumonia* (29.41%), 120 *Escherichia coli* (58.82%) and 24 *Pseudomonas aeruginosa* (11.76 %).

As shown in table 2, the isolated bacteria were identified using Gram reaction, colony characteristics and biochemical test.

Table 3 shows 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***
Zobo drink (n=5)	35	15	2
Egg roll (n=5)	20	20	5
Meat pie (n=5)	25	10	3
Moi-moi (n=5)	15	5	5
Okpa (n=5)	25	10	9
Total	120	60	24

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	Metallic 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	20mm	15mm	30mm	19mm
K. pneumoniae	15mm	18mm	22mm	15mm
P. aeruginosa	0mm	0mm	0mm	0mm

Discussion

The main aim of this study was to isolate, identify and determining the Antibiotic susceptibility of *Escherichia coli*, K. *pneumoniae* and *P. aeruginosa* from ready to eat food gotten from food vendors around COOU Uli campus, Anambra Nigeria.

A total of 204 bacterial pathogens were gotten from 220 culture positive samples from ready-to- eat foods sold around the COOU Uli campus, Anambra Nigeria. In total*E. coli* (58.82%) *(*a uropathogen that indicates feacal contamination) was the most predominant bacteria isolatedfollowed by *K. pneumoniae*(29.41%) and then *P. aeruginosa* (11.76%).

The detected high incidence of *E. Coli* in this study could be owned to its varied habitats which could be a cause of contamination for the food. Isolation of those organisms from the samples shows that these foods were exposed to unhygienic practices, too much personnel handling, use of poor-quality water during processing and undue exposure during retailing. Contamination with *K. pneumoniae* might be as result of poor personal hygiene, deposition of aerosols generated by coughing or sneezing by customers or vendors. Occurrence of *P. aeruginosa* in these foods might be owned to insufficient personal hygiene of vendors and improper cleaning of utensils leading to food layer deposition that favors the growth of biofilms

According to WHO (2008), there was 25% diarrhea in food borne illness caused by food infected with *E. coli*.

A study by Bhaskar *et al*.(2004) at Mangalore also demonstrated 93% bacterial contamination out of 60 street food samples tested. In a Mexican study a total of 103 taco dressings were sampled for *E. coli* and *Salmonella* sp. They found 44 (43%) contained *E. coli* and 5 (5%) *klebsiella*.

Among these foods, Zobo drinks revealed highest bacterial contamination (25.49%) followed by Egg roll (22.06%). These foods were on increase demand and hereafter made ready a bit earlier prior to ingesting and which was kept exposed to air contamination on waysides. 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*. (2010) shows some level of similarity with the findings from this study. A related study by Oluyege*et al*. (2009) 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%s) 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 revealed that some of the most popular types of ready-to-eat foods that are sold in canteens and cafeteria of Uli Campus are contaminated, which is unsafe for consumption. Some of the bacteria isolated especially *Escherichia coli* and *K. pneumonia* that are isolated in almost all 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.

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