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Original Research Article

THE INVESTIGATION OF SUSCEPTIBILITY OF ISOLATED BACTERIA TO ANTIBIOTIC IN RESTAURANTS IN GODFREY OKOYE UNIVERSITY

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Abstract: The investigation of susceptibility of isolated bacteria to antibiotic in restaurants in Godfrey Okoye University was carried in this study. Each of the isolated bacteria was tested with different antibiotic discs to its resistance or susceptibility to the antibiotic. Two species drug combination were used for Table 1, 2, 3, 4 and 5. Using kitchen A and Fork 1 utensil species/drug combination: (*Staphylococcus aureus/* Gentamycin, Septrin) and Fork 2 utensil, (*Staphylococcus aureus/* Ciprofloxacin, Rocephin, Streptomycin). In comparing with *Bacillus species/* Pefloxacin, Ampiclox, Zinnacep, Amoxacillin, Rocephin, Ciprofloxacin, Streptomycin, Erthromycin) and Fork 2 species/drug combination for *Bacillus species/*Pefloxacin, Ampiclox, Zinnacep, Amoxacillin, Erthromycin). The implication of using many antibiotic treatments on *Bacillus species* indicated that there is certain level of resistance of bacteria. This will enable individuals suffering from food toxicity to know the specific medication to use. The source of microbial contamination maybe from water and food sources. Therefore there is urgent need for improvement in the hygienic conditions of restaurants to reduce food poisoning this can be achieved through proper washing, drying, storing and sterilization of kitchen utensils.

Keywords: Antibiotic, E.coli, Kitchen Utensils, Hygiene, Environment, Sterilization

Introduction: Microorganisms live almost everywhere on earth where there is liquid water or even a tiny amount of moisture, including hot springs on the ocean floor, deep inside rocks within the earth's crust, on the human skin, in a cow's stomach, and inside a sponge used for washing dishes (Madigan and Martinko 2006). Bacteria are practically all invisible to the naked eye, with few extremely rare exceptions, such as *Thiomargarita namibiensis* (Schulz and Jorgensen, 2001). . They were originally described in extreme environments, but have since been found in all types of habitats (Robertson *et al.*, 2005). Eukaryotes are characterized by the presence of a nucleus, an

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organelle that houses the DNA. DNA itself is arranged in complex chromosomes. mitochondria are organelles that are vital in metabolism as they are the site of cellular respiration. Mitochondria are believed to have originated from symbiotic bacteria and have their own DNA, which is considered to be a remnant genome (Dyall *et al*,. 2004). Plant cells also have cell walls and chloroplasts in addition to other organelles. Chloroplasts produce energy from light by photosynthesis. Chloroplasts also are believed to have originated from symbiotic bacteria (Dyall *et al*., 2004). Most protozoan's are around 0.01–0.05 mm and are too small to be seen with the naked eye, but can easily be found under a microscope. However, forms that are up to 0.5 mm are still fairly common and can be seen with the unaided eye (Eugene *et al*., 2004). Items in the kitchen become contaminated by contact with contaminated people, foods, pets, or other environmental sources. The first and foremost suspect "gadget" in the kitchen is the human hand. Too often, people don't wash their hands before preparing food. More often, people don't wash their hands between handling possibly contaminated foods like meat and other foods that are less likely to be contaminated like vegetables. This "cross-contamination" is a leading cause of food borne disease.(Jiang and Rossen (1999); CDC and WHO, 2003). The intention of food safety is to prevent food poisoning, (the transmission of disease through food) and to maintain the wholesomeness of the food product though all stages of processing, until it is finally served. Therefore, one important task is to make sure dishes, spoons and cutlery are kept clean. This investigation intends to bridge the communication gap in relation to handling of kitchen utensils and to perform antibacterial susceptibility test on the isolates.

Material and Methods: For this study, the following materials were used: Peptone water, macconkey agar, blood agar, nutrient agar, normal saline, citrate medium, hydrogen peroxide, wire loop, incubator, crystal violet, acetone, bromothymol blue, lugos iodine, safranin, hand gloves, petri dishes, test tubes, whatman filter paper, antibiotics; *ciprofoxacin* (Cip,5 µg), *chloramphenicol* (Chl,30 µg), *gentamycin* (Gen,10 µg), *erythromycin* (Ery,15 µg) and *ampicillin* (Amp,10 µg), kovac's reagent (indole test), simmon's citrate agar (citrate utilization test). The items were sampled after the cleaning process was done for dishes, spoons, and cutlery. Samples were collected by means of swabbing using sterile cotton swab stick, moistened with normal saline, the samples were labeled indicating the location and date. All specimens were transferred to the microbiology laboratory within (1-3) hrs of the each specimen being taken and then inoculated into nutrient broth and MacConkey agar plates. The plates were incubated overnight at 37^{o}C (Vandepitte *et al*., 2003) and examined. Bacterial growth was checked after 24 - 48 hours. The growth was later sub cultured into Blood agar plates. The inoculated plates were incubated at 37°C for 24 - 48 hrs. Bacterial isolates were first differentiated by macroscopic examination of the colonies. The colonies were differentiated based on size, colour, pigmentation, elevation, surface texture, margin, haemolysis on blood agar plates, lactose fermentation on MacConkey agar and cloudiness on the nutrient broth. Several biochemical tests were also carried out to further identify the various bacterial isolates as described by Barrow and Feltham, (1993). A pure single colony grown overnight on blood agar was picked up using inoculating wire loop and placed in the tube containing normal saline. The mixture was then mixed thoroughly up and down using the Pasteur pipette to create a smooth suspension. The Whatman filter paper was used to prepare disks by punching using normal office two holes paper puncher. Prepared disks were placed in a sterile bottle and autoclaved for 15 minutes in 121 ºC at 15 Ibs. The disks were soaked in the following antibiotics: *ciprofoxacin* (Cip, 10µg), *amoxacillin* (Am 30 µg), *gentamycin* (Gen, 10

µg), *erythromycin* (Ery, 10 µg), *pefloxacin* (Pef 10 µg), *ampiclox* (Amp 30 based on Clinical and Laboratory Standards Institute (CLSI, 2013) recommendations. The disks were removed from the solution after absorbing and allowed to dry in the incubator before being placed onto the inoculums. The antibiotic susceptibility testing of the isolates were carried out using the Disk Diffusion Method on nutrient agar plate (CDC and WHO 2003). Petri dishes with nutrient agar were flooded with inoculums, allowed to distribute equally and to dry on the bench. Antibiotic disks were then applied on the surface of inoculated agar using sterile forceps. Finally the plates were incubated overnight at 37 ºC. Data were recorded based on the clear zones of inhibition. Zones were measured in millimeter (mm) using a ruler and compared to a standard interpretation chart based on performance standards for antimicrobial Disk Susceptibility Tests (CLSI 2013) used to categorize the isolates as susceptible, intermediate susceptible or resistant.

Results: The bacteria isolated from the kitchen utensils with their level of prevalence are shown in Table 1 below:

Table 1: Bacteria isolated with their level of prevalence

Organisms Identified	Degree of Growth	Prevalence (%)
Staphylococcus aureus	++++	29.5
Bacillus species	++++	29.2
Klebsiella species	++	9.2
Shigella species	+++	15.0
Salmonella typi	+++	9.0
Streptococcus pnemonia	++	6.6
Escherichia coli	+++	11.5

From the Table 1, *Staphylococcus aureus* had the highest prevalence of 29.5%, followed by *Bacillus species* with a prevalence of 29.2%. Other bacteria isolates had prevalence of 15% (*Shigella species*); 11.5% (*Escherichia coli*); 9.2% (*Klebsiella species*); 9% (*Salmonella typi*) and 6.6% (*Streptococcus pneumonia*).

Antibiotic susceptibility and resistance test was carried out on two isolated bacteria using antibiotic discs as presented in Table 2 below:

Table 2: Antibiotic test on bacteria isolated from utensils in kitchen A

Sample	**Kitchen A**				
	Staphylococcus aureus	***Bacillus species***		***Staphylococcus aureus***	***Bacillus species***
Fork 1	*Gentamycin* *Septrin*	*Pefloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Rocephin* *Ciprofloxacin* *Streptomycin* *Erthromycin*	**Fork 2**	*Ciprofloxacin* *Rocephin* *Streptomycin*	*Pefloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Erthromycin*
Plate 1	*Zinnacep* *Ampiclox*	*Pefloxacin* *Amoxacillin* *Rocephin* *Ciprofloxacin* *Streptomycin* *Erthromycin*	**Plate 2**	*Erthromycin* *Septrin* *Streptomycin* *Ciprofloxacin*	*Ampiclox* *Pefloxacin* *Zinnacep* *Amoxacillin* *Rocephin*

Spoon 1	*Streptomycin*	*Pefloxacin* *Ciprofloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Rocephin* *Ciprofloxacin* *Septrin* *Erthromycin*	**Spoon 2**	*Pefloxacin* *Ciprofloxacin*	*Ampiclox* *Zinnacep* *Amoxacillin* *Rocephin* *Ciprofloxacin* *Septrin* *Erthromycin* *Streptomycin*

From Table 2, ***Staphylococcus aureus*** *was treated with* ***gentamycin*** *and* ***septrin*** *on fork 1,* ***zinnacep*** *and* ***ampiclox*** *on plate 1 and* ***streptomycin*** *on spoon 1, while on fork 2,* ***ciprofloxacin****,* ***rocephin*** *and* ***streptomycin.*** *On plate 2,* ***ampiclox, pefloxacin, zinnacep, amoxacillin and rocephin****. Spoon 2 was treated with the following* ***antibiotic: ampiclox, zinnacep, amoxicillin, rocephin, ciprofloxacin, septrin, erythromycin and streptomycin****.*

Table 3: Antibiotic test on bacteria isolated from utensils in kitchen B

Sample	Kitchen B				
	Staphylococcus aureus	*Bacillus species*		*Staphylococcus aureus*	*Bacillus species*
Fork 2	*None*	*Pefloxacin* *Ciprofloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Rocephin* *Ciprofloxacin* *Streptomycin* *Septrin* *Erthromycin*	Spoon 1	*Ampiclox* *Ciprofloxacin* *Zinnacep*	*Ciprofloxacin* *Pefloxacin* *Rocephin* *Septrin* *Erthromycin* *Ampiclox*
Plate 1	*Ampiclox* *Pefloxacin* *Zinnacep*	*Ciprofloxacin* *Ampiclox* *Ciprofloxacin* *Streptomycin* *Rocephin* *Septrin* *Erthromycin*	Plate 2	*Septrin* *Ampiclox*	*Pefloxacin* *Ciprofloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Rocephin* *Ciprofloxacin* *Streptomycin* *Erthromycin*

For Table 3, fork 2 had no treatment for ***Staphylococcus aureus*** but spoon 1,plate 1 & 2 had treatment which ranged from ***ampiclox, ciprofloxacin, zinnacep*** ***(***spoon 1), ***ampiclox, pefloxacin, zinnacep*** ***(***plate 1) and ***pefloxacin, ciprofloxacin, ampiclox, zinnacep, amoxicillin, rocephin, ciprofloxacin, streptomycin, erythromycin*** (plate 2).

Table 4: Antibiotic test on bacteria isolated from utensils in kitchen C

Sample	Kitchen C				
	Staphylococcus aureus	*Bacillus species*		*Staphylococcus aureus*	*Bacillus species*
Fork 2	*Rocephin* *Septrin*	*Pefloxacin* *Ciprofloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Ciprofloxacin* *Septrin* *Erthromycin*	Fork 1	*Streptomycin*	*Rocephin*
Plate 2	*Streptomycin*	*Pefloxacin* *Ciprofloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Ciprofloxacin* *Erthromycin* *Septrin* *Rocephin*	Plate 1	*Pefloxacin* *Streptomycin* *Ampiclox*	*Ciprofloxacin* *Septrin* *Zinnacep* *Amoxacillin* *Ciprofloxacin* *Erthromycin*
Spoon 2	*Ciprofloxacin* *Zinnacep*	*Pefloxacin* *Ciprofloxacin* *Ampiclox* *Amoxacillin* *Rocephin* *Septrin* *Erthromycin* *Streptomycin*	Spoon 1	*Streptomycin* *Ciprofloxacin*	*Pefloxacin* *Ampiclox* *Zinnacep* *Amoxacillin* *Ciprofloxacin* *Erthromycin* *Septrin* *Erthromycin*

Table 4 shows a trend for isolated **Staphylococcus aureus,** fork 1 (**streptomycin),** fork 2 (**rocephin, septrin**); plate 1 (**pefloxacin, streptomycin, ampiclox**); plate 2 **(streptomycin)** while spoon 1 (**streptomycin, ciprofloxacin**) and spoon 2 (**ciprofloxacin, zinnacep**).

Table 5: Antibiotic test on bacteria isolated from utensils in kitchen D

Sample	KITCHEN D				
	Staphylococcus aureus	*Bacillus species*		*Staphylococcus aureus*	*Bacillus species*
Plate 2	*Zinnacep* *Rocephin*	*Gentamycin* *Rocephin* *Ampiclox* *Ciprofloxacin* *Septrin* *Erthromycin* *Rocephin* *Pefloxacin* *Amoxacillin* *Streptomycin*	Plate 1	*None*	*None*
Fork 2	*Septrin*	*Pefloxacin* *Streptomycin* *Zinnacep* *Erthromycin* *Rocephin* *Ciprofloxacin* *Amoxacillin* *Gentamycin* *Ampiclox*	Fork 1	*Ciprofloxacin* *Septrin* *Pefloxacin*	*Ampiclox* *Zinnacep* *Amoxacillin* *Rocephin* *Erthromycin* *Streptomycin* *Ciprofloxacin*
Spoon 2	*Gentamycin* *Ciprofloxacin* *Zinnacep*	*Pefloxacin* *Streptomycin* *Erthromycin* *Rocephin* *Amoxacillin* *Septrin* *Amoxacillin*	Spoon 1	*Ampiclox* *Gentamycin*	*Pefloxacin* *Ciprofloxacin* *Amoxacillin* *Streptomycin* *Rocephin* *Erthromycin* *Zinnacep* *Septrin*

Table 5 indicates a trend for isolated ***Staphylococcus aureus****, fork 1 (****ciprofloxacin, septrin pefloxacin****), fork 2 (****septrin****); plate 1* ***(none****); plate 2 (****zinnacep, rocephin)*** *while spoon 1 (****ampiclox, gentamycin****) and spoon 2 (****gentamycin, ciprofloxacin, zinnacep****).*

Discussion: The results from the study on the antibiotic susceptibility test showed a trend of increasing resistance rate in some patterns of treatment of kitchen utensil. This was in line with findings from CISI (2013), that there may be a clearly artificial change of susceptibility rates of species/drugs combination due to changes in AST guidelines. Two species drug combination were used for Table 1, 2, 3, 4 and 5. *Staphylococcus aureus* /*Bacillus species* which is in line with studies from Robertson *et al*., 2004 and Berdgoll (1989). Using kitchen A and Fork 1 utensil species/drug combination: (*Staphylococcus aureus/ gentamycin, septrin*) and Fork 2, (*Staphylococcus aureus/ ciprofloxacin, rocephin, streptomycin*). In comparing with *Bacillus species/ pefloxacin, ampiclox, zinnacep, amoxacillin, rocephin, ciprofloxacin, streptomycin, erthromycin*) and Fork 2 species/drug combination for *bacillus species/pefloxacin, ampiclox, zinnacep, amoxacillin, erthromycin*). The implication of using many antibiotic treatment on *Bacillus*

species indicated that there is certain level of resistance of bacteria which limits must not be exceeded. However, there should be a choice of antibiotic therapy for each infections from usage of such contaminated utensils. This can also be seen for other kitchen utensils of species/drug combination for Table 1, 2, 3, 4 and 5. The results of this study are in concordance with those of other authors demonstrating that susceptibility rates differ between species and environment (Dyall-Smith *et al*., 2004). In addition, it was observed that *gentamycin* susceptibility rates was only on *Staphylococcus aureus* isolate's collected from the kitchen utensils (fork 1 and spoon 2) decreased significantly on like other antibiotic. In conclusion, this study demonstrates that changes in susceptibility of antibiotic to isolated bacteria may differ between kitchen type (A, B, C, and D). Although, caution on clean environment for handling food and kitchen utensil must be emphasized, it is recommended that further studies are needed to assess the effect of the changes on other restaurant within Enugu metropolis and its environs (Bryan *et al*., 1995).

References

- Barrow, G. I and Feltham, R. K.A. (1993). Cowan and steel's manual for the identification of medical bacteria. 3rd Edition, Cambridge University Press, Cambridge, 331.
- Berdgoll, M.S. (1989). *Staphylococcus aureus*, Food Borne Bacteria Pathogen. *Int. J. Food Microbiol.* 26: 465-524.
- Bryan, F.L., Bartleson, C.A., Christopher, N. (1995). Hazard analysis in reference to Bacillus species in cantonese-style restaurants. *J. Food Protect*. p. 44.
- CDC/WHO. (2003). Guidelines for Environmental Infection Control in Health-care facilities. MMWR: 53(RRIO);1- 42.
- CLSI. (2013). Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobail susceptibility testing. Twenty-third Informational Supplement. M100-523. CLSI. Wayne, PA. USA.
- Dyall-Smith, M. L., Burns, D. G., Camakaris, H. M. (2004). Square bacteria grown in lab for the first time. *FEMS Microbiology letters, 238. 469- 473.*
- Eugene, W. N., Denise, G., Anderson, C., Evans, C., Roberts, J.R., Nancy, N., Pearsall, Martha, T. (2004). Microbiology. A Human Perspective. Fourth edition, McGraw-Hill Company pp. 635-636.
- Jiang, M and Rossen, X. (1999). The Food Encyclopedia of Photography. Journal of the Society of Imaging Science and Technology of Japan, p. 65.
- Madigan, M. and Martinko, J. (2006). Brock Biology of Microorganisms (13th ed.) Pearson Education. p. 1096.
- Roberton, S. P., Young, P. R., Stephens, D. (2005) Millimeter-wave substrate integrated waveguides and filters in photoimageable thick film technology. IEEE Transactions on Microware Theory and Techniques 53 (12), 3832 – 3828.
- Schulz, H. N and Jorgensen, B. B. (2001). Big bacteria. Annu. Rev Microbiol. 2001. 55:105 -137.
- Vandepitte, J., Engbaek., Rohner, P., Piot, P., Heuck, C.C., World Health Organization. (2003). Basic laboratory procedures in clinical bacteriology. *Journal of Bacteriology, p. 1938.*