Antibiotic Resistance Level in Bacterial Isolates from the Kakuri Industrial Drain In Kaduna, Nigeria.

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Abstract-

Bacterial isolates from the Kakuri drain in Makera District of Kaduna town were subjected to antibiotic resistance test using the Kirby-Bauer Disc-diffusion method. A total of thirty three (33) strains isolated from the drain were tested for their antibiotic resistance capacity using the M13, M14 and M51 Mastring Discs. The result obtained, indicated that the strains of Bacteria isolated from the drain are resistant to most of the tested antibiotics. Data obtained showed that all the isolates had multiple antibiotic resistance (MAR). All the strains showed resistance to Chloramphenicol (100%) at 25*u*g; Erythromycin(100%) at 5*u*g ; Tetracycline(100%) at25*u*g; Cotrimixazole (100%) at 25ug; Sulphatriad (80%) at 200*u*g Augmentin((100%) at 10ug ; Ampicillin (100%) at 10ug; among others, but were found to be sensitive to Streptomycin(40%) at 10*u*g on M13; sensitive to a lesser extent to Colostram sulphate(40%) at 25*u*g and Gentamicin(40%) at 10ug on M14. While on M51, disk , Strains were only noted to be sensitive to Gentamicin.

Introduction

The Makera-Kakuri axis is home to industries in Kaduna metropolis, as it accommodates most of the industries situated in Kaduna town. Several textile industries, an automobile plant, PAN (Peuquet Automobile Company),an arms factory with an electroplating unit and a superphosphate fertilizer company, among others are situated in this area. Treated wastewater from these industries is channeled in to large open drain that finally empties into river Kaduna. The makera district also houses several human settlements which also discharge sewage/domestic waste into the open drain. This result in the presence of antibiotics in the wastewaters in the drain; as residents consume antibiotics, and the hospitals dispenses antibiotics, while poultry farms and an abattoir also contribute to antibiotic load in the wastewater. These, and several other drugs are excreted and passed out as sewage, many of which are non-metabolized drugs and end up in the wastewaters of the drain. Poultry farms also contribute further to more antibiotics in the wastewater. Antibiotics in these wastewaters could alter the ecology of the environment and possibly give rise to antibiotic resistance (Ding and He, 2010). Many large bodies of water in the US share two major characteristics, that is, close proximity to residential communities, and receives industrially contaminated water-prone to antibiotic resistance (McArthur, 2015). Resistance to antibiotics may arise by cellular mutation or by acquisition of genetic elements in the form of plasmids or transposons. These plasmids also carry genes that provide resistance to heavy metals (Russel and Path, 2001).

The kakuri drain which receives these wastewaters finally empties its content into river Kaduna, a tributary of the river Niger. River Kaduna serves as the main source of portable drinking water for the inhabitants of Kaduna metropolis. The banks of the drain as well as the banks of the river Kaduna provide irrigation land for the residents who raise cash crops as lettuce, carrot, spinach, waterleaf, amongst others. These are crops consumed mostly in their raw form.

Materials and Methods

Wastewater samples for the experiment were collected from four designated points along the Kakuri drain. These points were labeled points C, D, E and F. The sampled water was then immediately conveyed to the laboratory for the assays. Kaduna town through which the drains passes, receives waste from homes, hospitals, industries and companies, poultry farms, abattoir etc, lies at an altitude of 10.20N and longitude 7.24E.

The Disk Diffusion Method

10 *u*l of the inoculum was dropped-inoculated on Muller Hilton plates and the prepared Disk pre-impregnated with a standard concentration of a particular antibiotic were then evenly dispersed and lightly pressed onto the agar surface. The plates where then inoculated at room temperature for 24/48 hrs. Clear zones of inhibition (>11.0mm) indicates the extent of the test organism"s ability to survive in the presence of the test antibiotic while absence of any zone of inhibition indicates resistance of the isolate to the test antibiotic. M13, M14 and M51 Disks employed giving a range of about 20 antibiotics. The antibiotics (content per disc used in the study) are- C : Chloramphenicol (25UG) : E: Erythromycin (5ug) : FC: Fusidic acid (10ug) : OX: Oxacillin (5ug) : NO:Novobiocin (5ug): PG: Penicillin G (1 unit): S:Streptomycin (10ug): T:Tetracycline (25ug) for M13. M14 content as reflected on M14 table and that of M51 as listed on M51 table. Because of convenience, efficiency and cost, the disk diffusion method was employed here as it is a widely used method for determining antimicrobial resistance in veterinary clinical and environmental microbiology.

The growth medium Mueller-Hinton agar was used and evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x 10^8 colony forming units per ml). Commercially prepared disks M13 M14 and M51 each of which were pre-impregnated with standard concentration of particular antibiotics were then evenly dispensed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse

outward from the disks, creating a gradient of antibiotic concentration in the agar such that the highest concentration would be found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc was observed. In test isolate that were susceptible to a particular antibiotic a clear area of "no growth" was observed around that particular disk.

The zone around an antibiotic disk that had no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. The zone was then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant (NCCLS, 1997). MIC measurement was not determined from this qualitative test, which simply classifies the isolate as susceptible, intermediate or resistant.

Result

TABLE 19 -ANTIBIOTIC RESISTANCE ASSAY –USING THE M13, M14, AND M51 MASTRING-S DISCS – 13 MASTRI USE OF M13 NG-S

N=RESISTANT P=SENSITIVE

USE OF M14 MASTRING-S

ANTIBIOTIC TESTS - NAME		ISOLATE A1		ISOLATE A2		ISOLATE A3									
AP			N		N		ST1	ST2		ST15		ST20	ST25	C1
KE		A1	A2	A3	C3	C5	1	5	N	0	ST15	ST20	ST25	C1
C	CO	N	N	P	N	N	P	N	N	P	N	N	N	N	N
E	GM	N	N	P	N	N	P	N	N	P	N	N	N	N	N
FC	S	N	N	P	N	N	P	N	N	P	N	N	N	N	N
OX		N	N	N	N	N	N	N	N	N	N	N	N	N	N
NO		N	N	N	N	N	N	N	N	N	N	N	N	N	N
PG		N	N	N	N	N	N	N	N	N	N	N	N	N	N
S		P	P	P	N	N	N	N	N	N	N	N	N	N	N
T		N	N	N	N	N	N	N	N	N	N	N	N	N	N

ST		P	N	N	N	N	N	N
T		N	N	N	N	N	N	N
TS		N	N	N	N	N	N	N

KEY
AP=AMPHICILLIN
10ug
KF=CEPHALOTHIN
5ug
CO=COLISTRIN SULPHATE 25ug
GM=GENTAMICIN 10ug
S=STREPTOMYCIN 10ug
ST=SULPHATRIOD 200ug
T=TETRACYCLINE 25ug
TS=COTRIMIXAZOLE 25ug

N=RESISTANT P=SENSITIVE

USE OF M51 MASTRING-S

ANTIBIOTIC NAME	ISOLATE	ISOLATE A1	ISOLATE A2	A3	ST15	ST20	ST25	C1
AP		N	N	N	N	N	N	N
NI		N	N	N	N	N	N	N
AUG		N	N	N	N	N	N	N
CIP		P	P	P	N	N	N	N
TM		N	N	N	N	N	N	N
CFX		N	N	N	N	N	N	N
GM		P	P	P	N	N	N	N
NA		N	N	N	N	N	N	N

KEY-
NI=NITROFURANTOIN- 50ug

AUG=AUGMENTIN- 30ug

CIP= CIPROFLOXACIN – 5ug

TM=TRIMETHOPRIN- 2.5ug

CFX=CEFALEXIN- 5ug

GM=GENTAMICIN- 10ug

NA=NALIDIXIC ACID- 30ug

N=RESISTANT P=SENSITIVE

Discussion-

The table for M13 Mastring-S shows the isolated bacterial strains as being resistant to some of the antibiotics on the disc. Strain A1 for instance, was found to be resistant to all the antibiotics except

Streptomycin, hence showing 87.5% resistance. Same result was obtained for strains A2 and A3. Strains ST15, ST20 and ST25 however, recorded 100% resistance to all antibiotics on M13 MASTRING-S. Strains A1, A2 and A3 were therefore found to be resistant to Chloramphenicol, Erythromycin, Tetracycline, Penicillin etc but sensitive to Streptomycin. While on M14, strain A1 was found to be resistant to Colistrin, sulphate, Gentamycin and streptomycin, recording 50% resistance. Strains A2 and A3 were susceptable to Cotrimixazole, Tetracycline, Ampicillin but intermediately susceptible to Cephalothin (less than 11mm), that is, 62.5% showed resistance to the tested antibiotics. Result from the M51 Disk shows that strains A1, A2 and A3 were all sensitive to Gentamicin(10ug) and Ciprofloxacin(5ug). However, they were found to show resistance to Ampicillin(25ug), Nitrofurantoin(50ug), Augmentin(30ug), Nalidixic acid(30ug), and Trimethoprin(10ug), amounting to 75% resistance. The result is consistent with findings by Bolaji et al 2011. Data obtained from related studies also suggests that Pseudomonas spp are equipped or are able to acquire wide range of antibiotic resistance mechanisms, and thus should be monitored as possible source of resistance genes (Luckiewicz et al., 2015).

The M51 Table therefore, shows the isolated bacterial strains as being susceptable to Ciprofloxacin and Gentamicin and resistant to Ampicillin, Augmentin, Cefalexin and the rest of the antibiotics on the Disc. Isolated bacterial strains were characterized as *Pseudomonas aeruginosa* from biochemical assays and molecular studies. Pseudomonas species are highly vasatile organisms with genetic and physiological capabilities that allow them to flourish in environments hostile to most pathogenic bacteria (Alice Prince, 1986). Pseudomonas spp. can survive in both low and high nutrition environments (Mera & Gerba, 2009). In addition to that, Pseudomonas can also help *Salmonellae* survive in this low nutrient environment (Warburton et al 1994). *Pseudomonas spp*. are also known to harbor multiple intrinsic and

acquired resistance genes, host several mobile genetic elements, and exchange them with other families of Gram negative bacilli like *Enterobacteriaceae* (Juan Nicolau & Oliver, 2011; Pfeifer et al 2010).

The high prevalence of antibiotic-resistant bacteria, identified as *Pseudomonas aeruginosa*, harboring diverse resistance traits could represent a potential health risk (DebMandal et al 2011).The spread and persistence of antibiotic resistance pose a severe threat to human health, yet there is still lack of knowledge about reservoirs of antibiotic resistant bacteria in the environment (Kittinger et al 2016). Carbapenemase production by Gram negative bacteria is one of the most concerning patterns of resistance encountered in patients today because it is associated increasingly with resistance to all recently marketed antibiotics (Karem et al, 2016). Emergence of resistance among the most important bacterial pathogens is recognized as a major public health threat affecting humans worldwide. Multidrug-resistant organisms have emerged not only in the hospital environment, but are often identified in community setting, suggesting that reservoirs of antibiotics-resistant bacteria are present outside the hospital (Munita and Arias 2015). The study of antibiotic resistance therefore helps predict future emergence of resistant bacterial strains and allow for strategies to forestall such occurrences.

Recommendation-

The presence of various strains of bacteria with multiple resistant to different antibiotics portrays the danger of drug resistance amongst persons living in that area. It is recommended that the government look into providing basic health infrastructure that will offer services to the people hence cut down on issue of self medication. This will help curtail the abuse of powerful antibiotics which could easily confer resistance to the bacterial pathogens.

Acknowledgement-

I Will like to acknowledge the assistance offered By, Kamaluddeen Kabir, Musili O Haroun and Akpai Mohammed in the conduct of the assays.

References-

Alice-Prince M. D (1986) Antibiotic Resistance of Pseudomonas Species, *Journal of Pediatrics*, vol. 108, issue 5, part 2, May 1986. Pp 830- 834.

Bolaji, A.S., Iromini, F.A., Adewoye,S.O and Ogunsola, O.A (2011) Antibiotic resistance pattern of bacteria spp. Isolated from hospital wastewater in Ede South Western, Nigeria. Pelgia Research Library. *European Journal of Experimental Biology*, 2011, 1 (4) : 66-71.

DebMandal, M., Mandal, S., and Kumar-Pal, N. (2011) Antibiotic Resistance Prevalence and Pattern in Environmental Bacterial Isolates. *The Open Antimicrobial Agents Journal*, 2011, 3, 45-52.

Ding, C., and He, J.,(2010) Effect of antibiotics in the environment on microbial populations. *Appl. Microbiol. Biotechnol*. 2010 Jul ; 87(3) : 925- 41.

Juan Nicolau, C., Oliver, A. (2010) Carbapenemases in Pseudomonas spp. Enferm. Infece, *Microbiol. Clin*. 28 (Suppl. 1), 19-28. 10. 1016 / SO213-005x(10) 7004-5. [PubMed][Cross Ref].

Karem, G, Chastre, J., Wilcox, M. H., and Vincent, J. L (2016) Antibiotic strategies in the era of multidrug resistance. Biomed Central. *Critical Care* 2016. 20: 136. DOI : 10. 1186/ S/ 13054- 016- 1320- 7. Ccforum.biomedcentral.com

Kittinger, C., Lipp, M., [. . .] and Zarfel, G. (2016) Antibiotic Resistance Patterns of Pseudomonas spp. Isolated from the River Danube. Front Microbiol, 2016 ; 7 : 586. *Frontiers in Microbiology*.

Luckiewicz, A., Kottarska, E., Artichowicz, W,. Tarasewicz, K., and Fudala-Ksiazek, S (2015) Antimicrbial resistance of Pseudomonas spp isolated from wastewater and wastewater-impacted marine coastal zone. *Environ, Sci. Pollut Res Int*. 2015 Dec ; 22(24) : 19823-34. Doi 10. 1007/s 11356-015 5098-y. Epub 2015 Aug 19. McArthur, J. V. (2015) UGA ecologist finds another cause of antibiotic resistance. Odum School of Ecology. University of Georgia. J Vaun McArthur, MCARTHUR@SREL.UGA.EDU.

Mera, K. D., and Gerba, C. P (2009) Risk Assessment of Pseudomonas aeruginosa in water. *Rev Environ. Contam. Toxicol*, 201, 71- 115. 10, 1007/ 978-1-4419- 0032 – 3 [Pub Med][Cross Ref] .

Munita, J. M. and Arias, C.A (2015) Mechanisms of Antibiotic Resistance. www.ncbi.nim.nih.gov. *Microbiol Spectr*. 2016 Apr 4(2) : 10. 1128/ microbiolspec. VMBF- 0016-2015.

NCCLS, (1997) National committee for clinical Laboratory standards. Zone diameter interpretive standards and equivalent minimum inhibitory concentration breakpoints for organisms other than Haemophilus spp., N. gonorrheae and S. pneumonia. Pensylvania, USA : NCCLS 1994.

Pfeifer, Y., Cullik, A., and Witte, W (2010) Resistance to Cephalosporins and Carbapenems in Gram-negative bacterial pathogens. *Int. J. Med. Microbiol*. 300, 371-379 10. 1016/J. ijmm. 2010. 04.005 [Pub Med][Cross Ref].

Russel, A. D. and Peth, FRC (2001) Mechanisms of Bacterial insusceptibility to biocides. *Am. J. Infect. Control*, 29 : 259- 261.

Warburton, D. W., Bowen B., and Konkle A. (1994) The survival and recovery of Pseudomonas aeruginosa and its effects upon Salmonella in water : Methodology to test bottled water in Canada. *Canadian Journal of Microbiology.* Vol. 40. Issue 12. Pp 987-992.