

Assessment of antibiotic resistance of bacteria from a lentic freshwater body in Iwo, Nigeria.

O.E. Atobatele¹ and A.A Owoseni^{2*}

¹Department of Biological Sciences (Zoology), Bowen University, Iwo, Osun State, Nigeria.

²Department of Biological Sciences (Microbiology), Bowen University, Iwo, Osun State, Nigeria.

ABSTRACT

Aims: Bacterial resistance to antibiotics has become a major public health concern, the aim was to study bacterial resistance to antibiotics in bacteria isolated from Aiba water sampled from 4 locations in 3 different months

Place and Duration of Study: Water samples were collected from Aiba reservoir Iwo and Isolation and characterization of bacteria was carried out at the Department of Biological Sciences, Bowen University, Iwo, Osun State between January 2013 and December 2013.

Methodology: Bacterial resistance to antibiotics was studied by collecting representative spatial and seasonal water samples from 4 locations and in 3 different months respectively. Isolation and identification of bacteria were carried out using standard isolation and biochemical methods. Antibiotic sensitivity testing was done for 8 antibiotics using the standard Kirby-Bauer disk diffusion method. Multivariate statistical analysis and plots were carried out using PAST software.

Results: One hundred percent (100%) of the isolates were resistant to cloxacillin followed by amoxicillin (87%), co-trimoxazole (78.3%) and augmentin (76%). Both gentamycin and ofloxacin recorded resistance of <10%. Thirty eight point five percent (38.5%) of the bacteria isolates were resistant to 4 antibiotics while only 11.5% exhibited mono-resistance.

Conclusion: The presence of high level multidrug resistance signifies a public health hazard.

Keywords: multidrug resistance; freshwater; Aiba; heterotrophic bacteria; water borne infections

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Antibiotic resistance is a phenomenon of increasing importance as demonstrated by the emergence of different international antimicrobial resistance surveillance programs. Since the first use of antimicrobial agents in the early 1940s, it has been known that bacteria carry mechanisms that allow them to resist antimicrobials. Bacterial resistance increased and became a worldwide human and veterinary health concern even with the introduction of the cephalosporins, fluoroquinolones among others [1].

Bacteria are capable of surviving in poor media such as water, floors and inanimate surfaces. Indicator bacteria have the ability to acquire and disseminate resistance that could be transmitted to pathogenic or zoonotic bacteria. Following antibiotic therapy, antibiotic residuals and resistant bacteria are shed into the environment via manure [2,3] which can be washed off via rain water into water bodies. Plasmids and other mobile genetic elements such as transposons, insertion sequence elements and integrons may play a role in facilitating transfer of antibiotic resistance genes to various bacterial communities in the

34 environment [4,5]. Transport of these antibiotic resistance bacteria into groundwater and
35 streams is not unlikely and it may be detrimental if the antibiotic resistance plasmids are
36 ultimately mobilized to opportunistic human pathogens [6].

37 Aiba Reservoir being the second oldest impoundment of Osun River Basin was created for
38 the provision of potable water with fisheries development as an ancillary benefit to Iwo and
39 surrounding communities. In and around the reservoir are activities such as fishing, washing
40 of domestic wares and automobiles, bathing and fetching of water for construction and
41 domestic purposes [7].

42 These different contamination sources can lead to the introduction of multidrug resistant
43 bacteria. Hence this paper was designed to study the antibiotic resistance patterns of
44 bacteria isolated from the Aiba Reservoir water body.

45

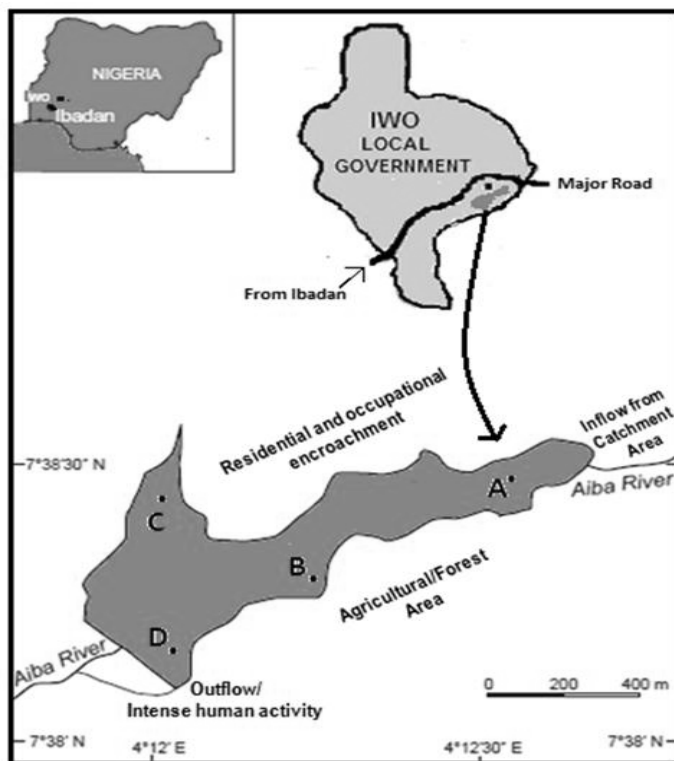
46 2. MATERIAL AND METHODS

47

48 2.1 Sampling

49 Water samples from Aiba Reservoir were collected from four locations (A, B, C and D) within
50 the reservoir (Figure 1) and in three months (October, March and June) representing
51 different seasonal periods. Water samples were collected from each point using a sterile
52 500ml polyethylene bottle. The water samples were transported to the laboratory within one
53 hour of collection and kept in the refrigerator until ready for analyses [7].

54



55

56

57 Figure 1: Map of Aiba Reservoir showing sampling locations.

58 Key:

59 A: North-east, near source of inflow (upstream), little human activity (occasional washing/bathing).

60 B: South-east, shares border with land used for agricultural activities, minimal direct human
61 interference

62 C: North, rapid residential and occupational encroachment, food-vendor, carpentry, mechanic
63 workshops, washing of vehicles and motorcycles

64 D: South, close to outflow (downstream), landing site for fishermen, high human activity-swimming,
65 bathing, domestic washing, and removal of fish intestines.
66

67 2.2 Bacterial isolation and identification

68 Heterotrophic plate counts and total coliform counts were carried out according to
69 standardized methods [8]. Bacterial culture and isolation was carried out using the serial
70 dilution methods as described by Brown [9]. Different dilutions were pour plated on plate
71 count agar for Total heterotrophic plate count and MacConkey agar for Total Coliform count,
72 these were carried out in triplicates. Incubation was carried out at 37°C for 24h for the
73 heterotrophic bacteria and at 37°C for 48h for the coliforms. Distinct colonies were picked
74 and restreaked onto fresh agar plates until pure cultures were gotten. The pure cultures
75 were kept on nutrient agar slants and kept at 4°C.

76 Isolates were characterized morphologically and biochemically. Gram staining, spore
77 staining, colonial morphology, indole, Methyl red, Voges-Proskauer, catalase, citrate, starch
78 and casein hydrolysis were carried out to characterize the isolated bacteria [10]. Bergey's
79 manual of systematic bacteriology [11] and the manual of identification of medical bacteria
80 [12] were also used.

81

82 2.3 Antibiotic sensitivity testing of isolates.

83 Antibiotic susceptibility tests were done using the standard Kirby-Bauer disk diffusion
84 methods [13]. One milliliter of an overnight actively growing broth culture adjusted to contain
85 1×10^6 cfu/ml of each bacterial isolate was introduced into a Petri dish and 20 ml of molten
86 Mueller Hinton agar added. Antibiotic sensitivity discs (Abtek Biologicals Ltd.) containing
87 different antibiotics namely nitrofurantoin (200µg), augmentin (30µg), ofloxacin (5µg),
88 tetracycline (10µg), gentamycin (10µg), chloramphenicol (10µg), ampicillin (25µg), nalidixic
89 acid (30µg), and amoxicillin (10µg), erythromycin (25µg), cloxacillin (10µg), co-trimoxazole
90 (25µg), and streptomycin (25µg) were placed on the solidified agar surface. The plates were
91 incubated overnight at 37°C. The relative susceptibility of each isolate to each antibiotic was
92 shown by a clear zone of inhibition. Susceptible strains showed clear zones around the
93 antibiotic discs [14, 15].

94

95 2.4 Statistical analyses

96 Multivariate analysis and plots were carried out using PAST software [16]. Principal
97 Components Analysis (PCA) was determined using the correlation matrix and varimax
98 rotation method; while PCA biplot was done to determine the relationship between bacterial

99 isolates and sensitivity to antibiotics. Hierarchical Cluster Analysis (HCA) was done using
 100 Ward's method.

101

102 3. RESULTS AND DISCUSSION

103

104 Seventy-nine (79) species belonging to sixteen (16) genera were isolated from the water
 105 samples from Aiba Reservoir. This is as shown in Table 1. The *Bacillus* genus was the most
 106 isolated while *Acinetobacter*, *Edwardsiella*, *Listeria*, and *Proteus* were the least isolated with
 107 2 species each.

108

109

110 Table 1: Genera and number of isolated bacteria from Aiba Reservoir water

S/N	Bacteria genera	Number isolated(per genus)
1	<i>Bacillus</i>	31
2	<i>Escherichia</i> , <i>Enterobacter</i>	5
3	<i>Aeromonas</i> , <i>Flavobacterium</i> , <i>Staphylococcus</i>	4
4	<i>Alcaligenes</i> , <i>Citrobacter</i> , <i>Corynebacterium</i> , <i>Klebsiella</i> , <i>Lactobacillus</i> , <i>Streptococcus</i>	3
5	<i>Acinetobacter</i> , <i>Edwardsiella</i> , <i>Listeria</i> , <i>Proteus</i>	2

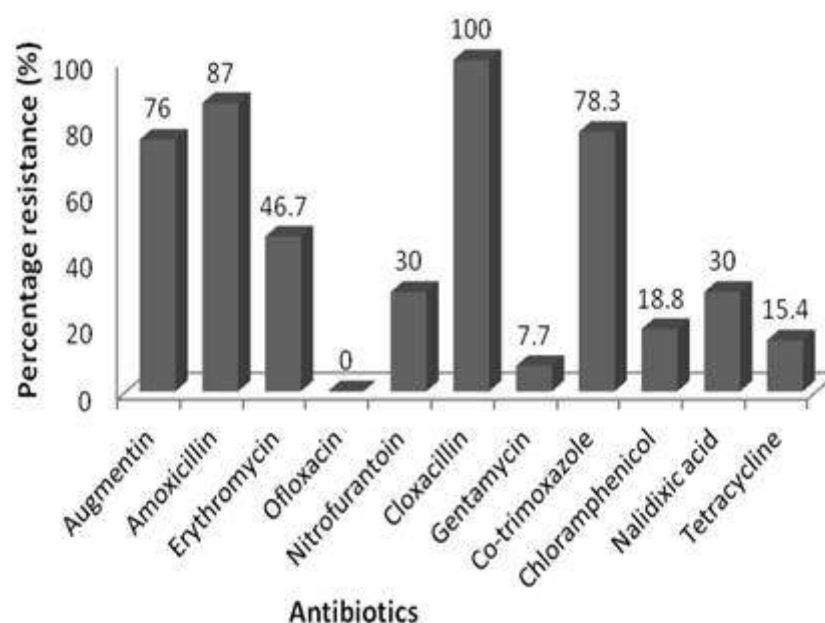
111

112 Figure 2 shows the percentage resistance of the isolated genera to the tested antibiotics.

113 There was 100% resistance to cloxacillin, amoxixillin (87%), co-trimoxazole (78.3%) and

114 Augmentin (76%). Ofloxacin and gentamycin had 0% and 7.7% resistance respectively.

115



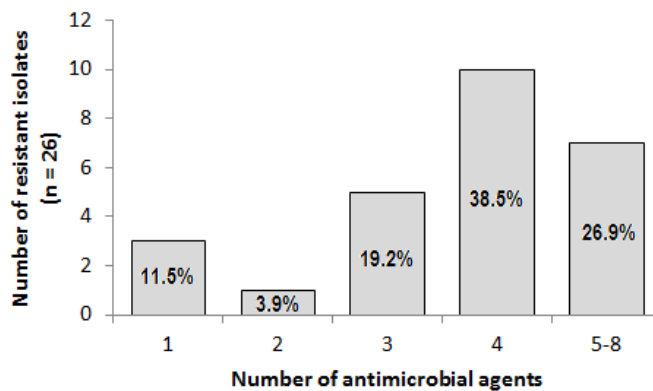
116

117

118 Figure 2: Percentage resistance of isolated bacteria genera from Aiba Reservoir to different
 119 antibiotics

120

121 The percentage of mono- and multi resistant bacteria isolated from Aiba reservoir is shown
 122 in Figure 3. Thirty-eight point five percent (38.5%) of the resistant bacteria were resistant to
 123 4 antibiotics while 26.9% was resistant to between 5 and 8 antibiotics. Only 11.5% was
 124 mono-resistant. In all, 88.5% of the resistant bacteria were multi resistant as shown in Figure
 125 3.
 126
 127
 128
 129
 130



131
 132
 133

134 Figure 3: Percentages of mono- or multi-resistant bacteria isolated from Aiba Reservoir
 135 water. (Percentage of resistant bacteria isolates is enclosed within each bar).
 136

137

138 Table 2 gives the patterns of multiple resistance in the isolated bacteria to the different
 139 antibiotics. Sixty-five point four percent (65.4%) of the isolates were resistant to both
 140 amoxicillin and augmentin while 53.9% was resistant to amoxicillin-augmentin and co-
 141 trimoxazole simultaneously. The pattern/combination that showed the lowest resistance
 142 pattern was amoxicillin, augmentin, ampicillin and cloxacillin.
 143

144

145 Table 2: Patterns of multi-resistance in isolated bacteria

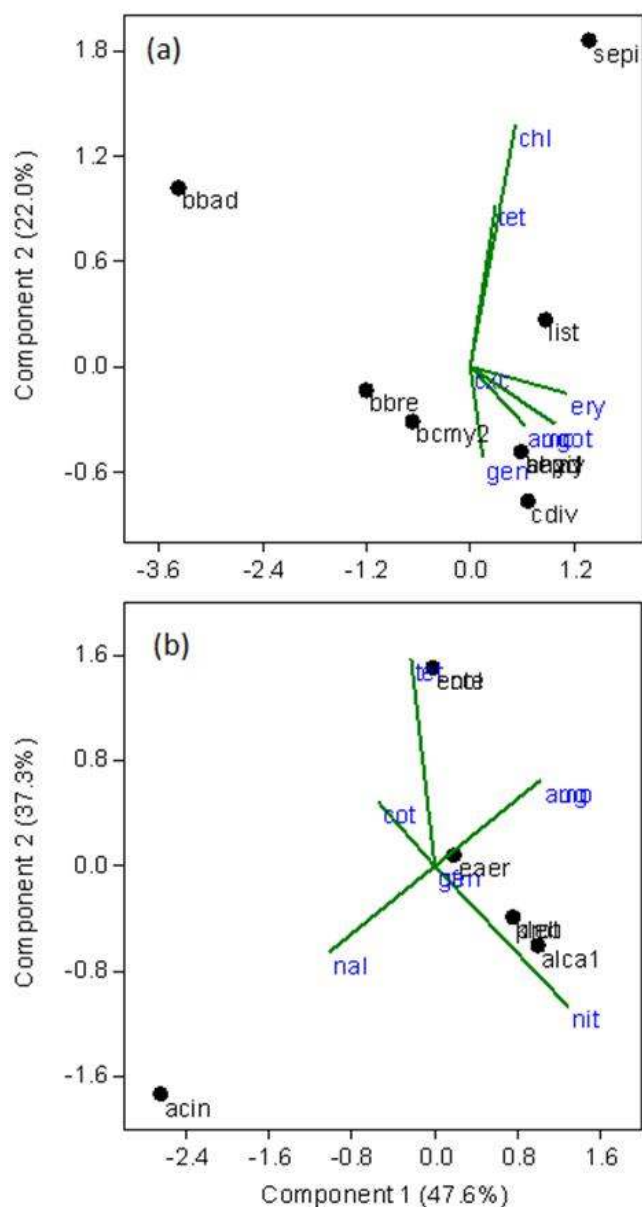
Antibiotic multi-resistance pattern	Resistant strains (%)
*amx-cot-aug-tet	7.7
amx-aug	65.4
amx-aug-cot	53.9
amx-tet	11.5
amx-aug-amp-cxc	3.9
amx-aug-cxc	34.6
cxc-cot	30.8
cot-cxc-gen	7.7

146 *amx: amoxicillin, cot: co-trimoxazole, aug: augmentin, tet: tetracycline, cxc: cloxacillin, gen:
 147 gentamycin

148

149

150 Figure 4 shows the Principal Component Analysis (PCA) biplots for Gram positive (a) and
 151 Gram negative(b) bacterial isolates resistant to the tested antibiotics. The PCA biplots show
 152 that the isolated bacteria were grouped into three and the antibiotics were grouped into two.
 153
 154
 155

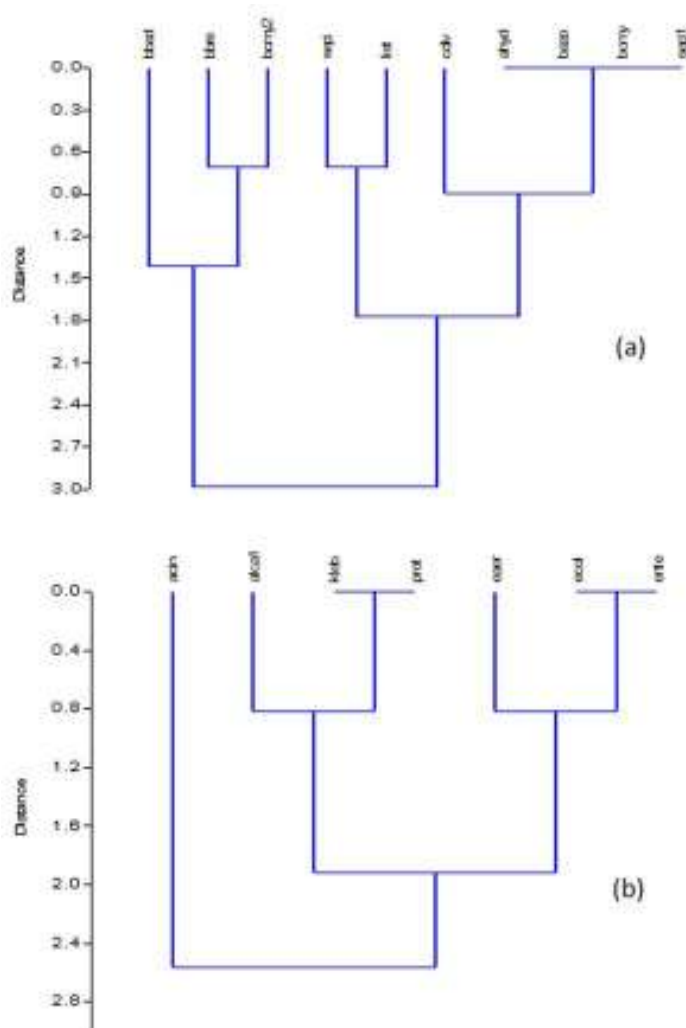


156
 157
 158 Figure 4: Principal Component Analysis (PCA) biplots for Aiba Reservoir water (a) Gram
 159 positive and (b) Gram negative bacterial isolates resistant to tested antibiotics.

160

161 The dendrograms of cluster analysis (Figure 5) show that the resistance patterns of the
 162 *Bacillus brevis* and *Bacillus cereus* subsp *mycoides* was similar to *Bacillus badius*. They are
 163 members of a 60-phenon. The second group of *Staphylococcus* and *Listeria* species have a
 164 very similar resistance pattern (80-phenon) while the third group have a 70-phenon.
 165 Resistance patterns of groups 2 and 3 were fairly similar (40-phenon) but there were no
 166 similarities between the resistance patterns of group 1 and the other two groups. The
 167 isolates in the group 2 were all resistant to chloramphenicol, tetracycline and gentamycin. It
 168 was also observed that the *Bacillus* group was resistant to the same group of antibiotics.

169



170

171

172 Figure 5: Dendrogram of Cluster Analysis of resistance of (a) Gram positive and (b) Gram
 173 negative bacterial isolates from Aiba Reservoir water using Ward's method.

174 The high populations of viable heterotrophic bacteria encountered are typical of water bodies
175 that receive organic pollutants. Similar high populations of heterotrophic bacteria have been
176 reported [17]. It has been suggested that freshwater habitats harbour bacterial species [18].
177 Gastrointestinal microbial pathogens and many waterborne disease outbreaks are caused
178 by intake of contaminated drinking water; in addition, drug resistant bacteria have been
179 reported in surface water and groundwater [19]. This is a major public health concern as
180 drug resistant bacteria could be transferred to humans via consumption of contaminated
181 drinking water which then contributes to the spread and persistence of antibiotic resistance
182 bacteria in the general population and environment. This study showed the presence of
183 mono and multi drug resistant bacteria in the surface water of Aiba Reservoir, the major
184 drinking water source in Iwo, Osun State.

185 Resistance patterns of isolated bacteria were cloxacillin (100%), amoxicillin (87%),
186 cotrimoxazole (78.3%) and augmentin (76%). These drugs are sold easily across the
187 counter and most antibiotics can be sold without a prescription. Amoxicillin is bacteriolytic
188 and affects bacterial cell wall synthesis. It is a β -lactam antibiotic hence susceptible to β -
189 lactamase producing bacteria. It is often combined with clavulanic acid, a β -lactamase
190 inhibitor and thus increases its effectiveness. This combined drug is sold as augmentin. It
191 was observed in this study that resistance in amoxicillin was higher than that in augmentin. It
192 was also observed that many of the isolates showed a multidrug resistance pattern. Sixty-
193 five point four percent (65.4%) of the resistant bacteria were resistant to amoxicillin and
194 augmentin simultaneously while 53.9% were resistant to amoxicillin-augmentin-co-
195 trimoxazole. The highest multi-resistance pattern shown was resistance to 4 antibacterials
196 by 38.5% of the isolates while 26.9% of the isolates were resistant to between 5 and 8
197 antibacterial agents. This should be a cause for concern by public health analysts.

198 Quinolone resistance is important in human and veterinary medicine, since the situation
199 becomes more serious when it is demonstrated that a bacteria resistant to one quinolone
200 can be resistant to all antimicrobials of the same family [1]. In this study, all the isolates were
201 susceptible to ofloxacin tested and only 7.7% of the isolates were resistant to gentamycin. It
202 was observed in this study that most of the drugs to which resistance developed can be
203 administered orally and are cheap, hence easily accessible. The injection form of
204 gentamycin is most common hence the probable reason for very low resistance to it.

205 *Bacillus* was the most isolated organism in this study. They have the important feature of
206 producing spores that are exceptionally resistant to unfavourable conditions. Although most
207 *Bacillus* spp. are harmless, a few are pathogenic to humans and animals. *Bacillus cereus*
208 causes food poisoning and bacteraemia in immuno-compromised patients as well as
209 symptoms such as vomiting and diarrhea. *Bacillus anthracis* causes anthrax in humans and

210 animals. Species are often detected in drinking water supplies, even supplies treated and
211 disinfected by acceptable procedures. This is largely due to the resistance of spores to
212 disinfection processes [20]. *Aeromonas* spp. are normal inhabitants of fresh water and
213 occur in water, soil and many foods, particularly meat and milk. Some species cause
214 infections in humans, including septicemia. Some also cause respiratory tract infections and
215 gastrointestinal illnesses [21]. *Aeromonas hydrophila* produces cytotoxic enterotoxins
216 contributing to maladies such as travelers' diarrhea and has shown an increase of antibiotic
217 resistance [22]. *Citrobacter* rarely is associated with infections in humans. However, it has
218 been hypothesized that because of its low infection rate and virulence, *Citrobacter* could be
219 a reservoir for antibiotic resistance genes [23].

220 *Escherichia coli* is present in large numbers in the normal intestinal flora of humans and
221 animals where it generally causes no harm. However, in other parts of the body, *E. coli* can
222 cause serious disease, such as urinary tract infections, bacteraemia and meningitis.
223 Waterborne transmission of pathogenic *E. coli* has been well documented for recreational
224 waters and contaminated drinking water [24]. Normal intestinal flora are a reservoir for
225 resistance genes, the prevalence of resistance in commensal *E. coli* and some other
226 bacteria is a useful indicator of antibiotic resistance in bacteria in the community.
227 Commensal bacteria with reduced susceptibility to ceftriaxone have been isolated from
228 drinking water and feed from dairy and poultry farms [25]. Yang and co-workers [25]
229 concluded that ceftriaxone resistance was frequently associated with resistance to multiple
230 antibiotics.

231 Chloramphenicol resistance was modest at 18.8% in this study. Chloramphenicol
232 susceptible *Escherichia coli* was isolated from sewage but it was found that *E. coli* isolated
233 from livestock was resistant to chloramphenicol [26]. It was observed that some soil and
234 water environmental samples harboured significantly high numbers of drug-resistant bacteria
235 [27]. Tetracycline resistance was the most prevalent at all sites and resistance frequencies
236 ranged from 47% to 89% of total bacteria. Similar results have been reported from bacteria
237 in the environment [28, 29].

238 The PCA biplots show that the isolated bacteria were grouped into three and the antibiotics
239 were grouped into two. It was also observed that the *Bacillus* group was resistant to the
240 same group of antibiotics. The dendrograms of cluster analysis show that the resistance
241 patterns of the *Bacillus brevis* and *Bacillus cereus* subsp *mycoides* was similar to *Bacillus*
242 *badius*. They are members of a 60-phenon. The second group of *Staphylococcus* and
243 *Listeria* species have a very similar resistance pattern (80-phenon) while the third group
244 have a 70-phenon. Resistance patterns of groups 2 and 3 were fairly similar (40-phenon) but
245 there were no similarities between the resistance patterns of group 1 and the other two

246 groups. The isolates in the group 2 were all resistant to chloramphenicol, tetracycline and
247 gentamycin. These three are antibiotics that work against protein synthesis. Majority of the
248 other bacteria were resistant to antibiotics with the modes of actions of cell wall lyses.
249 The biplots and dendrograms also show that the resistance pattern of *Acinetobacter* was not
250 similar to the remaining isolates. The group 2 isolates of *Alcaligenes*, *Klebsiella* and *Proteus*
251 had resistance patterns that were very similar (80-phenon). *Klebsiella* and *Proteus* were
252 totally similar (100-phenon). The 3rd group consists of *E. coli*, *Enterobacter* sp (100-phenon)
253 and *Enterobacter aerogenes*. Groups 2 and 3 had resistance patterns that were fairly similar
254 (40-phenon) but very different from that of *Acinetobacter*. Similarity in resistance patterns of
255 the enterobacteria species could be due to horizontal gene transfer. This has been reported
256 [30]. It is possible that many of the multi-drug resistant bacteria isolated in this study
257 acquired their resistance via horizontal gene transfer which could be found on R-plasmids
258 [30]. Application of manure/sludge is a major route of introduction of antibiotic resistant
259 bacterial genes into water bodies. This has been reported [31, 32]. Another problem of
260 resistant bacteria is the transference of resistance genes from harmless to pathogenic
261 bacteria or to humans interacting with that aquatic environment.

262
263

264 **4. CONCLUSION**

265
266

267 In conclusion, the multiple drug resistance exhibited by the isolated organisms in this study
268 is a threat to public health. Their presence signifies a public health hazard and could
possibly lead to waterborne diseases.

269
270

271 **ACKNOWLEDGEMENTS**

272
273

274 This work was supported by the Bowen Learned Conference Fund under Grant reference
275 number [BUI/CRLCF/01/10].
276

AUTHORS' CONTRIBUTIONS

Author OEA carried out the water sampling, performed the statistical analysis, and read the draft. Author AAO managed the Laboratory analyses and wrote the first draft of the manuscript. Both Authors designed the study, managed the literature searches and read and approved the final manuscript.

REFERENCES

1. San Martin B, Campos L, Bravo V, Adasne M, Borie C. Evaluation of antimicrobial resistance using indicator bacteria isolated from pigs and poultry in Chile. *Intern J Appl Res Vet Med*. 2005;3(2):171-78
2. Hamscher G, Pawelzick HT, Hoper H, Nau H. Different behaviour of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. *Environ Toxicol Chem*. 2005;24:861-68.
3. Sapkota AR, Lefferts LY, McKenzie S, Walker P. What do we feed to food-production animals? A review of animal feed ingredients and their potential impacts on human health. *Environ Health Persp*. 2007;115(5):663-70.
4. Bennet PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *Brit J Pharmacol*. 2008;153:5347-5357.
5. Heuer H, Kopmann C, Binh CTT, Top EM, Smalla K. Spreading antibiotic resistance through spread manure: characteristics of a novel plasmid type with low %G plus C content. *Environ Microbiol*. 2009;11:937-49.
6. Rahube TO, Yost CK. Characterization of a mobile and multiple resistance plasmid isolated from swine manure and its detection in soil after manure application. *J Appl Microbiol*. 2012;112(6):1123-33.
7. Atobatele OE, Owoseni AA. Distribution and diversity of bacteria in a small tropical freshwater body (Aiba reservoir) in Iwo, Osun state, Nigeria. *Nat Sci*. 2012;10(12):92-7.
8. APHA/AWWA/WEF. Standard methods for the examination of water and waste water. 20th edition. American Public Health Association/American Water Works association. Water Environment Federation, Washington DC, USA. 1998;235-37.
9. Brown AE. Benson's Microbiological Applications. 9th edition New York: McGraw-Hill;2005.
10. Pollack RA, Findlay L, Mondschein W, Modesto RR. Laboratory Exercises in Microbiology. 2nd edition. John Wiley and Sons Inc. USA. 2002;51-53.
11. Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's manual of systematic bacteriology; Vol.2. Baltimore: Williams and Wilkins;1986.
12. Barrow GI, Feltham RKA, editors. Manual for the identification of medical bacteria 3rd edition. Cambridge: Cambridge University Press;2004.

13. Quinn PJ, Carter ME, Markey B, Carter GR. Clinical veterinary microbiology. London: Wolfe Publishing, 1994;95-102.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45:439.
15. Owoseni AA, Onilude AA. Antibiotic Sensitivity and Sequence amplification Patterns of Genes in Multidrug Resistant Enterobacteria Isolates from Processed Foods in some West African Countries. Pol J Microbiol. 1966;60(4):309-16.
16. Hammer O, Harper DAT, Ryan PD. PAST, Paleontological Statistics software package education and data analysis. Paleontol Electron. 2001;4(1):9.
17. Olayemi AB. Bacteriological water assessment of an urban river in Nigeria. Intern J Environ Health Res. 1994;4:156-64
18. Yannarell AC, Kent AD. Bacteria, Distribution and Community structure. Encyclopedia of inland waters. 2009;3:201-10.
19. Tao R, Ying G-G, Su H-C, Zhou H-W, Sidhu JPS. Detection of antibiotic resistance and tetracycline resistance genes in Enterobacteriaecae isolated from the Pearl Rivers in South China. Environ Pollut. 2010;158:2101-09.
20. Bartram J, Cotruvo JA, Exner M, Fricker C, Glasmacher A, editors. Heterotrophic plate counts and drinking water safety: the significance of HPCs for water quality and human health. WHO Emerging issues in Water and Infectious diseases series. London: IWA Publishing; 2003.
21. Borchardt MA, Stemper ME, Standridge JH. *Aeromonas* isolates from human diarrheic stool and groundwater compared by pulsed-field gel electrophoresis. Emerg Infect Dis. 2003;9:224-28.
22. Sha J, Kozlova E, Chopra A. Role of various enterotoxins in *Aeromonas hydrophila*-induced gastroenteritis: Generation of enterotoxin gene-deficient mutants and evaluation of their enetrotoxic activity. Infect Immun. 2002;70(4):1924-35.
23. Pepperell C, Kus J, Gardam M, Humar A, Burrows L. Low virulence *Citrobacter* species encode resistance to multiple antimicrobials. Antimicrob Agents Chemother. 2002;46(11):3555-60.
24. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev. 1998;11:142-201.
25. Yang H, Dettman B, Beam J, Mix C, Jiang X. Occurrence of ceftriaxone-resistant commensal bacteria on a dairy farm and a poultry farm. Can J Microbiol. 2006;52:942-50.
26. Sayah R, Kaneene J, Johnson Y, Miller R. Patterns of antimicrobial resistance observed in *Escherichia coli* obtained from domestic-and wild-animal faecal samples, Human septage and surface water. Appl Environ Microbiol. 2005;71(3):1349-1404.

- 383 27. Esiobu N, Armenta L, Ike J. Antibiotic resistance in soil and water environments.
384 Intern J Environ Health Res. 2002;12(2):133-44.
385
- 386 28. Bayne S, Blankson M, Thirkell D. Enumeration and speciation of group D
387 streptococci from above and below a sewer outfall, their susceptibilities to six
388 antibiotics and a comparison with clinical isolates. A Van Leeuw. 1983;49:299-310.
389
- 390 29. Blanco JE, Blanco M, Mora A, Blanco J. Prevalence of bacterial resistance to
391 quinolones and other antimicrobials among avian *Escherichia coli* strains isolated
392 from septicemic healthy chickens in Spain. J Clin Microbiol. 1997;35:2184-2185.
393
- 394 30. Zwenger SR, Gillock ET. Bacteria isolated from sewage influent resistant to
395 ciprofloxacin, chloramphenicol and tetracycline. J Environ Sci Heal A. 2009;44:123-
396 29.
397
- 398 31. Macauley JJ, Qiang Z, Adams CD, Surampalli R, Mormile MR. Disinfection of swine
399 wastewater using chlorine, ultraviolet light and ozone. Water Res. 2006;40:2017-26.
400
- 401 32. Kim S, Aga DS. Potential ecological and human health impacts of antibiotics and
402 antibiotic-resistant bacteria from wastewater treatment plants. J Toxicol Env Heal B.
403 2007;10(8):559-73.
404