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MULTI-DRUG RESISTANT *AEROMONAS* SPECIES IN ANNELIDA: AN EVIDENCE OF PATHOGEN HARBOURING LEECH IN RECREATION WATER NEXUS OF OGHARA NIGERIA ENVIRONS

Bright E. Igere[134]*, Blessing B. Igolukumo[1], Eduamodu C.[4], Emmanuel O. Odjadjare[124]

[1]Department of Microbiology and Biotechnology, Western Delta University, Oghara, Delta State. Nigeria.
[2]Environmental, Public Health and Bioresource Microbiology Research Group, Department of Basic Sciences, Benson Idahosa University, PMB 1100, BeninCity, Nigeria.
[3]Institute of DNA/RNA Research; Lahor Research Laboratories P.M.B 4, 121 old Benin-Agbor Road, Ikpoba Hill, Benin City, Edo State, Nigeria.
[4]Department of Biochemistry and Microbiology, University of Fort Hare, South Africa
*Correspondence: 201710685@ufh.ac.za; ibe22002@yahoo.com; Tel.: +27-733491868; +2348038792425.

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ABSTRACT

Aeromonas infections have shown diverse complications in management due to the multiple antibiotic resistance observed amongst its members. The origin and habitat of its resistance development yet remain vague. The present study depicts Leech and its infested recreational water as a reservoir of multidrug-resistant Aeromonas hydrophila. Skin swabs of recreational water bathers before (Bb)/after bathing (Ab) (250 each) and 250 leeches (Hirudomedicinalis) were collected from recreational water sites. Standard Microbiological and Molecular biology methods were applied for isolation and characterization. Two hundred and forty-five (98%)Bb specimen, showed a negative growth oforganisms, 84% (210) of the Ab specimen harboured presumptive Aeromonas species, while 100% (250) Leeches specimen harboured Aeromonas species. The Polymerase Chain Reaction (PCR) of 16SrRNA gene detection confirmed all (465/100%) isolates as Aeromonas species while 13.6% (63) were further delineated as Aeromonas hydrophila. The antibiogram showed 45(97.82%) resistance to the β-lactam antibiotic and other antibiotic groups. The PCR detection of resistant markers, virulent and plasmid profile of isolates reveals conjugative plasmid mediation, lip (123/97.6%), act (104/82%), hhly (93/73%) genes and Bla_{ampC} gene, $Bla_{TEM,}$ gene and Bla_{SHV} gene. Observation of pathogens with similar multiple antibiotic-resistant gene-profile both in the bathers'skin swab and gut of leeches indicates origin/habitat, association and suggests the gut of leeches as breeding habitat for the pathogen. This is an emerging public health concern that associates specifically the environment and human superficial infections.

Keywords: *Aeromonas hydrophila*, leeches (*Hirudomedicinalis)*, recreational water, bathers, virulent genes, antibiotic-resistant markers, PCR, plasmid profile

INTRODUCTION

Recreational centres are valuable community asset with essential qualities that influence social-economic values, health and environmental benefit for a community. It serves as a thriving hub of activities that provides needs for families, youths, senior citizens, civic organizations and physical activities (Forrester, 2014). It also has rooms for social clubs and academic programs to complement the

school experience for students (Forrester, 2014). However, there are several concerns associated with the usage of recreational water which are grouped into abiotic and biotic concerns. The abiotic includes drowning and activity-related injury (heat and cold) whereas the biotic is mainly microbial/biological contamination (Vermont, 2012). Other relative sources of concern include boating, recreational vehicle holding tanks, pumping stations, damaged sewer lines. (Vermont, 2012). Infections have been the result on post occurrences of major abiotic concerns, traced to poor portable toiletsandmicrobial/ biological contamination of water bodies. These aforementioned areas of concern tend to enhance faecal pollution and proliferation of aquatic organisms, affecting water quality (Igere *et al*., 2020a; Sharma *et al*., 2003).

One such free-living bacteria associated with aquatic nexus and has caused hazards in recreational water usage is *Aeromonas hydrophila* as listed by WHO(WHO, 2003). Its study has the potential to determine safety and sustainability of recreational water usage. Another organism of related concern is the Annelida {hirudinid leeches (*Hirudo medicinalis*)}, whose digestive-tract microbiota reveals a symbiont that was taxonomically grouped using 16SrRNA gene sequences as *Aeromonads* (Busing *et al*., 1953). Interestingly, other members of the genera (*Aeromonas*) were isolated from the midgut of *Culex quinquefasciatus* (*A.culicicola*), and *Aedes aegyptii* mosquitoes (*A. veronii*), suggesting that the genus colonize the gut or tract of blood-feeding organisms (Huys and Cnockaert, 2005; Pidiyar, 2002).

Aeromonads are Gram-negative, facultative non-spore-forming anaerobe, rod-shaped, which are autochthonous but distributed in aquatic environments (Daskalov, 2006). The genus is mainly psychrophiles and mesophiles of soil and water origin implicated in different diseases of warm and cold-blooded animals. Generally, members of the genus are characterized into three biochemical sub-groups **(***Aeromonas hydrophila***,** *Aeromonas sobria,*and *Aeromonas caviae***),** but recently, newer species have been added (Janda and Abbott, 2010). Most infections of *Aeromonas* spp. are acquired through contact with contaminated water or ingestion of foods in various "farm to table" operations (Dos Silva *et al.*, 2019; Pougnet*et al*., 2018; Khajanchi*et al*., 2010). The bacterium is also implicated in cellulitis or infected wound cases due to traumatized injury in the aquatic environment (Janda and Abbott 2010; Khajanchi*et al.*, 2010; Goñi-Urriza*et al.*, 2000). It has also been reported in severe cases of bacteriemia, dysentery and diarrhoea (Dos Silva *et al*., 2019; Mudaliar *et al*., 2019; Pougnet*et al*., 2018; Khajanchi*et al.*, 2010; Albarral*et al*., 2016). Its presence has also been reported in animal food sources, eye respiratory tract, sepsis, multiple antibiotic resistance and other deep-seated infections of public health concern. (Ali *et al*., 2014; Korkoca*et al*., 2014; Enany*et al*., 2011). *A. hydrophila* has been listed in the Contaminant Candidate List of EPA and the Environmental Protection Agency Method 1605 has confirmed its enumeration and detection in water (the United States Environmental Protection Agency, 2001) as a microbial contaminant.

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Its resistance to β-lactam (penicillin, ampicillin, carbenicillin, and ticarcillin) has been used in their phenotypic characterization and detection of resistant markers. (Bosch *et al*., 2019; Mudaliar *et al*., 2019; El-ghareeb*et al*., 2019; Ahned*et al*., 2018; Awan, *et al*., 2009). However, most *Aeromonas* species show susceptibilities to antibiotic members such as second and third-generation cephalosporins, piperacillin and azlocillin quinolones, tetracycline, aminoglycosides, chloramphenicol and trimethoprim-sulfamethoxazole (Awan, *et al.*, 2009; Sen and Rodgers, 2004). However recent studies reveal resistant dissemination in the ecosystems which necessitates their microbial diverse nature (Igbinosa *et al*., 2017). It is no doubt that most environmental activities and water reuse have contributed to pollution, bacteria proliferation, as well as the spread of antibiotic-resistance (Igere *et al*., 2020a; Igbinosa *et al*., 2015). Some of such resistant phenotypes have not been traced/associated to any specific origin hence this study aims to characterize antibacterial resistance markers, ARGs, virulent gene markers, biofilm formation and plasmid profile of *Aeromonas hydrophila* isolated from Oghara environs in Nigeria as an approach for source tracking resistance acquisition amongst the pathogen and its relative environmental health concern.

MATERIALS AND METHODS

Sample collection

The specimen was collected from three recreational centres within Ethiope West local Government area of Delta State, a total of 250 skin swab specimens each of apparently healthy visitors of the recreational centre after bathing (Ab), and before bathing (Bb) as a control, while 250 leeches (*Hirudo medicinalis*) were collected from the flow-in-water and flow-out-water during the study between February and September.

Fig 1: Photomicrograph of collected leech (*Hirudomedicinalis*)

Bacterial Isolation

Swab specimens and leeches were cultured onto alkaline peptone water (APW) (Merck, Germany), MacConkey agar (Biotech, Rajasthan, India), blood agar base (Biotech, Rajasthan, India) and incubated at 37^{o}C. Leeches were allowed

to thrive in the APW for 24 h, feed with fresh blood to release their oesophagal bacteria density into the medium. Bacteria growth on APW were subcultured onto Starch Ampicillin Agar (SAA) and incubated at 37^{o}C overnight (Khamesipour*et al.*, 2014). After 24h incubation, cultures of observable growth were then flooded with about3 ml of Gram's (Lugol's) iodine solution. Yellow to slightly brownish colonies was reported as amylase positive colonies. Other presumptive test include motility test, Gram-reaction, cytochrome-c oxidase test, methyl red test, indole production test, catalase test, citrate utilization test, urease test, glucose fermentation and arginine dihydrolase test, ornithine decarboxylase test, ONPG test, H_2S from cysteine, Acetylmethylcarbinol production (Voges-Proskauer test), L-arabinose utilization. APW tubes that produce reactions and acid butt after 24 h incubation were considered presumptively positive or negative for the specified biochemical reaction for *A. hydrophila* as previously described by Khamesipour*et al.,(*2014). A strain of *A. hydrophylia* ATCC7966 was a gift from Dr Odjadjare AEMREG South Africa in 2015 was used as the positive control.

Motility Test

Isolated bacterial colonies were stab-inoculated onto the centre of test tubes containing a semi-solid LB medium (LB base, 0.3% agar) and incubated at 30^{o}C for 24 h to assess motility.

Quantitative determination of Biofilm formation

With minor modifications from the methods of Jiang and his colleagues (Jiang *et al.*, 2015), a quantitative analysis of biofilm formation by the isolates and transformant cell was performed using a 96-well microtiter plate (polystyrene). Isolates and transformant strains were suspended in 10ml tryptic soy broth (TSB) (Biolab, Merck, Gauteng) and incubated at 37^{o}Covernight. This treatment was done for isolates, transformants and controls (positive and negative). Cloudy cultures were diluted 1:100 onto freshly prepared TSB medium and standardized using 0.5 McFarland. The prepared cells were washed with phosphate-buffered saline solution(PBS; pH 7.2) and resuspended in TSB medium, 100 µl aliquots of each standardized culture were then added to pre-cleaned and washed 96-well microtiter plate and incubated overnight at 30^{o}Cwhile transformants cells were incubated for two days in a stable position. The liquid media was discarded as adherent cells were carefully washed on each well of the microtitre plate using phosphate-buffered saline (PBS) and plates were allowed to dry at room temperature for one hour. Well of plates were then stained with crystal violet (1%) for 20 min. Thereafter, the cells were washed with sterile distilled water and allowed to air dry at room temperature. Crystal violet dye-stained adherent cells were resuspended in 150 µl absolute ethanol, vortex for 3-5 mins and optical density (OD) of each well reading obtained at 570 nm using a bioanalyser microtitre plate reader (Biolit-205, Germany). Biofilm formation were reported as negative (non-producing) when OD_a equals OD_b, + (moderate producing) when $OD_b \leq 0.1$ and ++ (strong producing) when $OD_b \geq 0.1$. The control wells containing only TSB broth were used as negative controls while those containing the *Aeromonas hydrophila* ATCC 7966 as

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a positive control. (Jiang *et al*., 2015). OD_a is an initial optical density of cell-free eluted solvent containing Crystal violet-stained non-adherent cells

while OD_b is the optical density of eluted solvent containing Crystal, violet stained adherent cells.

DNA extraction (genomic and Plasmid DNA)

Genomic DNA used in the PCR analysis (40ng/µl) was extracted from bacterial broth cultures using QiaAmp mini spin Kit and eluted using AE buffer according to the manufacturer's protocol (QIAGEN, Germany), while plasmid DNA was extracted using the QiaAmp Plasmid Mini Kit (QIAGEN, Germany).

DNA yield and purity

The concentration (purity) of total DNA from each organism was determined after extraction using an Absorbance at 260 nm and 280 nm. The optical density (OD) or appropriately called purity of DNA values were calculated as the ratio of absorbance at 260 nm to absorbance at 280 nm.Pure DNA, had an A260/280 ratio of 1.7-1.9 (QIAamp Blood kit, 2007). Isolated DNA samples having values within this range were used. Following the determination of the OD260/280 value of the samples, the concentration of the total DNA was adjusted to 100ng/µl and exactly 3 µl of the DNA samples were used as a template for polymerase chain reactions (PCR). The quality and concentration of fewer numbers of samples were assessed by DNA amplification, agarose gel electrophoresis and visualization using Alpha-imager. Initial trials were done during which reaction conditions were optimized and the reaction programme was standardized.

Curing Process

Isolates were subjected to SDS curing agent according to a modified method of Yang *et al*. (2008) and susceptibility test carried out on cured isolates. The results were compared with the pre-curing antibiogram.

Conjugation/transformation assays

The recipient cell used is (*Escherichia coli* K-12 DH1) which is a gift from Prof. Agbonlahor DE. The donor cell (*Aeromonas hydrophila*) and the recipient strain were both grown separately on sterile tubes of 30ml LB medium fortified with $MgCl_2$/CaCl (Merck, Modderfontein) at 30 °C for 24 h, cultures were then mixed in equal volumes (1:1) in a sterile tube and incubated for about 20 hrs or till mid-exponential phase which allows a possible transfer or sharing of plasmids. The mixed culture from the tube was then plated on Mueller Hinton agar plates which contain cefuroxime (CXM-30µg), ceftazidime (CAZ-30µg), gentamicin (Gm-30 µg), tetracycline (Tet-30µg) and Augmentin (AUG-30µg), and incubated for 24 h at 30 °C. The transconjugants were tested for the presence of the transferred genes by PCR analyses. Plasmid DNA of donor isolates and recipient transconjugants were extracted using the QiaAmp Plasmid Mini Kit(QIAGEN, Germany). Experiments were performed in triplicate to ensure reproducibility. The MIC values of gentamicin, ceftazidime, augmentin, tetracycline and cefuroxime of both donor and recipient cells were determined according to CLSI (CLSI, 2015). The frequency of transferred plasmid was

determined as the ratio of transconjugants (recipients that were able to receive plasmid DNA from a donor) to donor cells. (Osinska*et al*., 2016)

Transformation assay

The recipient cell (Escherichia coli K-12 DH1) was prepared by adding 0.5 ml aliquot of overnight broth culture onto 20 ml of freshly prepared nutrient broth in a flask, incubated at 37 °Cin an incubator attached to a rotating shaker (100rpm) until cell density is $5x10^7$ cells per ml. (an absorbance of 0.55 at 550nm is produced using a spectrophotometer). Culture is then chilled in ice for 10minutes and centrifuge at 3000xg for 15minutes at 4 °C. The cell pellet is further washed in 10ml of ice-cold 0.1M $MgCl_2$, and 5ml of ice-cold 0.1M $CaCl_2$, with simultaneous ice incubation and finally resuspended in 5ml ice cold Calcium Chloride. An aliquot of 200µl of the prepared cells (competent cells) was then added to 40µl of plasmid DNA extract according to Sambrook *et al*. (1989) and Danifol consult. (2005).Selection of the transformants was done using agar plate containing antibiotics which are reported following CLSI guidelines of the donor cells. Positive and negative controls were included to verify the experimental reproducibility of recipient cells.

Antimicrobial susceptibility tests

Antibacterial susceptibility testing of isolates, recipient positive/negative controls and transformant bacteria were conducted using the agar disk diffusion method as described by CLSI (Clinical Laboratory Standards Institute, 2015) guidelines. The antimicrobial disks used as supplied by both Oxoid, Abtek (England) and Mast Diagnostics, Merseyside, the United Kingdom via Davies Diagnostics (Pty) Ltd, 141 Oak Avenue, Ferndale, Randburg, 2194, Gauteng, South Africa (www.daviesdiagnostics.co.za) were: cotrimoxazole (Cot-25 µg), amoxycillin (Amx-25 µg), spectinomycin (Spec-100µg), cefixime (Cef-5 µg), cloxacillin (CXC-30µg), penicillin (Pen-30 µg), augmentin (Aug-30µg), ofloxacin (Ofx-5µg), gentamicin (Gm-30µg), nalidixic acid (Na-30µg), nitrofurantoin (Nit-200µg), tetracycline (Tet-30 µg), ampicillin (Amp-30 µg), cefotaxime (CTX-30 µg), ceftazidime (CAZ-30 µg), chloramphenicol (C-30µg), imipenem (IMI-30 µg), kanamycin (K-30 µg), meropenem (Mem-10 µg), cefuroxime (CXM-30 µg), streptomycin (Str-30 µg), sulfamethoxazole/trimethoprim (TS-25 µg), ciprofloxacin (CIP-5 µg), erythromycin (Ery-10 µg). The antibiogram of Isolates pre- and post-transformation were recorded and interpreted using CLSI guidelines (CLSI, 2015).

Multiple Antibiotic Resistance Index (MARI)

The MAR index was determined using the method of Odjadjare *et al*. (2012) with few modification where NI^ra = number of used antibiotics that reveal resistance by isolate; N^ab = total number of antibiotics used against individual isolates.

$MARI = NI^ra/N^ab$

Detection of genus and specie specific genes using PCR

The various presumptive isolates were subjected to specie specific gene detection for the specific identity of the isolates using PCR. Primer pairs applied and thermal conditions were as previously

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employed by various investigators as indicated (Stratev*et al*., 2016; Nawaz *et al*., 2010) were purchased from Inqaba Biotechnical Industries (Pty) Ltd. (Hatfield Pretoria, South Africa). Briefly, ***16SrRNA*** primer sets of **F-5′GGG AGT GCC TTC GGG AAT CAG A′3** and **R-5′TCA CCG CAA CAT TCT GAT TTG′3**, with amplicon size of 356bp, *A.hydrophyla* specie specific primer sets of ***aerA*** **F-5′ CAA GAA CAA GTT CAA GTG GCC A ′3** and **R- 5′ACG AAG GTG TGG TTC CAG T′3** with an expected amplicon size of 309 bp, haemolysin gene primer sets ***ahh1*****F-5′GCC GAG CGC CCA GAA GGT GAG TT′3** and **R- 5′GAG CGG CTG GAT GCG GTT GT′3** with expected amplicon size of 130 bp (Stratev*et al*., 2016), the cytotoxic enterotoxin gene primer sets act F-5'**GAGAAGGTG ACCACCACCAAGAACA**'3 and R-5'**AACTGACATCGGCCTTGAACTC**' 3 with the size of 232 bp (Nawaz *et al*., 2010) and lipase gene primer sets Lip F-5'**ATCTTCTCCGACTGGTTCGG**'3 and R-5'**CCGTGCCAGGACTGGGTC TT**'3 with a size of 382bp (Sen and Rodgers, 2004). The PCR mix used was as follows; A final volume of 25 µl PCR reaction which consisted of 12 µl GoTaq ®G2 green master mix supplied in 2X Green GoTaq®G2 reaction buffer containing pH: 8.5, dNTPs {400 µM each of dATP, dGTP, dCTP and dTTP}, 3 mM $MgCl_2$ and GoTaq®G2 DNA polymerase at the optimal concentration for efficient PCR amplification as specified by Promega Corporation USA (www.promega.com) was used. Primer concentration of 0.5 µM with forward primer 1µl, reverse primer 1µl, nuclease-free water 8µl, bacterial DNA extract of 3µl, (Shah *et al*., 2009). The cycling condition used on the thermal cycler (PTC-200 Thermocycler, MJ Research, Germany)was as follows; an initial denaturation temperature of 94°C for 5 min, 40 cycles of 94°C for 45 sec., 59°C for 45 sec., 72°C for 30 sec., and final elongation at 72°C for 10 min while act gene PCR condition was initial denaturation temperature at 94ºC for 5 min, with 40 cycles of 30 sec at 94ºC denaturation, annealing 30 sec at 55ºC, elongation of 30 sec at 72ºC and a final elongation of 7 min at 72ºC. Amplicons of various genes were separated on 1.5% agarose gel electrophoresis (electrophoresis machine CLS-AG100, Warwickshire, United Kingdom) for 50 mins at 100 Volt. The gels were stained with 2 µl per 50ml of 0.5 mg/L ethidium bromide and photographed in a gel documentation system. (AlphaimagerTM 3400, USA). A Thermo Scientific O'GeneRuler 100 bp ladder which was supplied by inqaba Biotech South Africa was used to determine the molecular size of amplicons (Hafez *et al*., 2018; Wang *et al*., 2003).

Detection of antibiotic Resistant genes using PCR

Antibiotic-resistant genes were detected using the methods as described by Hugh G Griffin and Annette M Griffin (2000). PCR of the resistant genes was conducted using thePeltier-200 Thermal Cycler. The following primer sets were used: for ***bla$_{TEM}$*** (535bp) F: 5'-**AGGAAGAGTATGATT CAACA**-3', R: 5'-**CTCGTCGTTTGGT ATGGC**-3';(Wroblewska *et al*., 2004);for *bla*$_{SHV}$(930bp)F: 5'-**GGGTTATTCTTAT TTGTCGC**-3', R: 5'-**TTAGCGTTGCC AGTGCTC**-3'**(**Amita Jain and Rajesh

Mondal, 2008**)**; and***bla**ampC*** (882bp) F: 5'-**GGTATGGCTGTGGGTGTTA**-3', R: 5'-**TCCGAAACGGTTAGTTGAG**-3' (Yang *et al*., 2008). A PCR mix of 25µl and one Taq 2X master mix with standard buffer which contains 1.8mM $MgCl_2$, 20mM Tris-HCl,22mM NH_4Cl, 22mM KCl, 0.2mM dNTPs, 5% glycerol, 0.06% IGEPAL CA-630, 0.05% Tween 20, and 25Units/ml of one Taq DNA polymerase. (New England Biolabs; www.neb.com) was obtained. A 10µM concentration of each primer pair, nuclease-free water and DNA extract as template was added to a Taq 2X master mix and prepared in a final volume of 25µl.Amplification was performed using Peltier thermal cycler PTC-200 Thermocycler (MJ Research, Germany) with cycling conditions as follows. initial denaturation at 94ºC for 3 min followed by 35 cycles each of denaturation at 94ºC for 30 sec, annealing at 50 ºC, 52 ºC, 54 ºC respectively at one minute for each primer. Initial extension proceeded at 72^0C for 2 min and a final extension at 72^0C for 8 min. The amplified products were electrophoresed on an ethidium bromide (0.5 mg/ml) stained 1.5% agarose gel for 40 min at 100 volts of edvotek system and gels were visualized in a gel documentation system (AlphaimagerTM 3400, USA). A Thermo Scientific O'GeneRuler100 bp ladder which was supplied by Inqaba Biotech South Africa was used to measure the molecular weights of amplified products.

RESULTS

Bacterial Isolation

A total of seven hundred and fifty specimens were collected between February and September which spans in equal proportion of skin swab of recreational water bathers at the recreational centres and harvest of black Leech (*Hirudomedicinalis*). Specimen collected from the subject before bathing showed no observable growth of study organism rather other organisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* etc.The results show that 98% (245) of such specimen collected before bathing, does not yield observable growth of the organism in the study, 84% (210) of the swab specimen collected after bathing harbored A*eromonas specie*, 100% (250) *Leeches* specimens harbored *Aeromonas specie*, while 13.6% (63) of the total isolates were PCR confirmed as *Aeromonas hydrophila* (Table 1). The various isolated organisms reported the following morphological and biochemical results; Amylase positive, motile, Gram-negative, cytochrome oxidase-positive, gas production positive from glucose, glucose fermentation positive, salicin fermentation positive, H_2S positive, arginine dihydrolase positive, ornithine decarboxylase negative, ONPG positive and L-arabinose utilization positive.

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Table 1: Showing the distribution of *Aeromonas* amongst the collected specimen

Specimen Code	Numb. Collected Before Bathing	After Bathing	Numb positive *Aeromonasspp* Before(%)	After(%)	Numb negative *Aeromonasspp* Before (%)	After (%)	Numb Positive Biofilm	Numb of Confirmed *A. hydrophila*
RecA (AmenA)	94	92	3 (3.2)	80 (87.0)	91 (96.8)	12 (13)	15/215	13
RecB (LegitO)	80	86	2 (2.5)	72 (83.7)	78 (97.5)	14(16.3)	10/215	9
RecE (KeldoE)	76	72	Nil	58 (80.6)	76(100)	14 (19.4)	8/215	5
Leeches	250		250 (100)		Nil		51/250	36
Total	**750**		**465**		**285**		**84**	**63**

Virulence associated genes of the isolates

The various members of the *A.hydrophila*, (63 isolated/confirmed members) were subjected to virulent determinative test and were observed to harbor the cytotoxic enterotoxin (*act)* gene (57/63; 90.5%), lipase (*Lip*) gene (59/63; 93.7%), haemolysin (*hhyl*) gene (47/63; 74.6%) in addition to diverse virulent phenotypes, biofilm formation, motility, extra-chromosomal DNA (plasmid) acquisition and multiple antibiotic resistance.

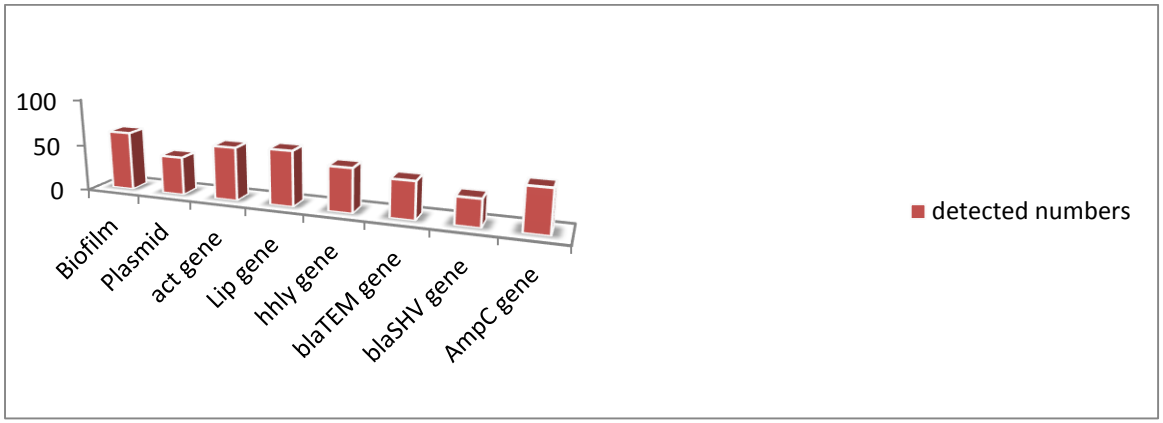

Fig 2: Numbers of occurring detected antibiotic resistant genes and virulent determinants

Antibiogram of isolates

Following the repeated reports on the occurrence of antibiotic-resistant pathogens in the globe, there are limited chances of antibiotic choice for therapeutic management. The isolates were subjected to a battery of antibiotics as listed earlier and their antibiogram and percentage sensitive is as shown in Table 2. The antibacterial susceptibility profile of isolates reveals resistance to; penicillins (100%), gentamycin (77.6%), streptomycin (74.0%)], cloxacillin (96.3%), cefixime (67.1%), ampicillin (98.3%), cefuroxime (52.9%), ceftazidime (70.8%), cefotaxime

(45.0%) and tetracyclines (88.2%)]. However, susceptibility was observed amongst nitrofurantoin (76.6%), and fluoroquinolones [nalidixic acid (81.1%), ciprofloxacin (89.5%) and ofloxacin (77.6%)] members. The multidrug resistance profile of the isolates in this study revealed that 272 (58.5%) of the total isolates were multidrug-resistant and their MARIndex at 0.57 as shown in Table 3.

Curing Analysis

An observable change in the pre-treatment and post-treatment antibiogram was recorded in the curing process which indicates that plasmid was responsible for some of the resistance observed amongst the potential pathogen. The plasmid extraction experiment shows that 65.1% (41) harboured plasmid of size range 1kbp –20kbp which has made the isolate that harboured plasmid produces a differing antibiogram from the pre-curing experiment (Table 4, fig 2). However, 22.2% (14/63) of the multidrug-resistant isolates were chromosomally mediated, as the curing report shows a mirror prototype of resistant profile as observed before the curing experiment.

Transformation and Conjugation

The results on mating the wild organism (*A.hydrophila*) with the recipient strain shows that 27(42.9%) of the isolates had transferable plasmids with resistant markers as follows; Pen^r, Tet^r, Aug^r,Cxc^r, Gen^r, Str^r, Chl^r and amp^r. The transformation analysis further confirms that these plasmids were harbouring multiple resistant markers both from isolates of skin and leeches. These pathogens possess similar resistant markers, virulent genes, extra-chromosomal DNA (plasmid) of similar band size and resistant genes. This is an indication that the organism originates from a similar parent or may have in their existence had contact and shared extrachromosomal DNA components. It was observed that 23.8% of the isolated plasmids could not be transferred to the standard recipient strain. This may be as a result of/or inability of plasmid reception by the standard strain and the rate of plasmid loss during sample analysis.

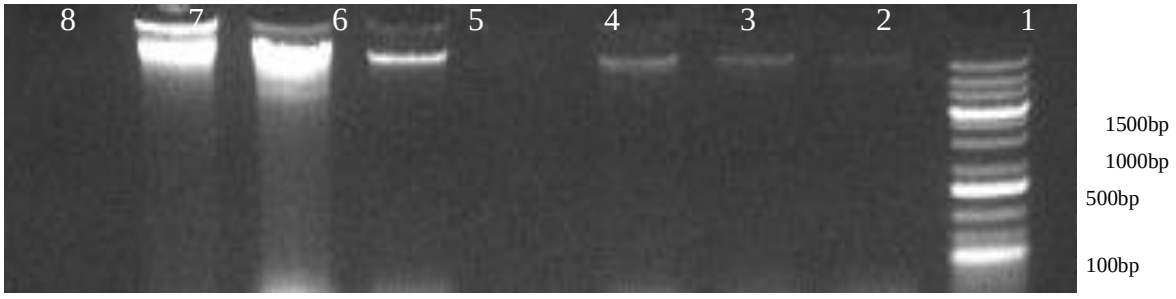

Figure 3: Plasmid profile of multiple drug resistance *Aeromonas species* analyzed with 0.8% agarose gel electrophoresis. L is 100bp-1.5kb ladder (molecular marker). Lanes 1, 2, 3, 5, 6, and 7 are positive for plasmid, lanes 5, 6, 7 had multiple plasmid bands with size $\geq$1.5kbp while Lanes 4 and 8 were negative for plasmid acquisition.

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Table 3: Antibiogram and Resistance Markers of Isolates after Curing

Bacterial Strain	Number of Isolates (%)	Resistant Markers	MAR Index detected	Resistant genes
Aeromonas hydrophila N=63	251(54.0)	CAZ^r,CTX^r,$AUG^r$$PEN^r$,$CXC^r$, CEF^r,CXM^r, CRX^r	0.57	bla_{TEM}, bla_{shv}
	272 (58.5)	PEN^r,GEN^r, STR^r ,KAN^r, CXC^r, AMX^r, AMP^r, AUG^r, SPC^r	0.52	$bla_{AmpC}bla_{TEM}$
	322 (69.2)	PEN^r, AUG^r,CXC^r,AMX^r, AMP^r,	0.39	$bla_{AmpC}bla_{TEM}$, bla_{shv}
	284 (61.1)	CAZ^r, CTX^r,CEF^r,CXM^r, CRX^r	0.39	bla_{TEM}, bla_{shv}
	254 (54.6)	STR^r,KAN^r,GEN^r, SPC^r	0.35	bla_{AmpC}
	30 (6.5)	NAL^r,CIP^r,OFL^r,	0.22	Nil
	391 (84.1)	$MERO^r$,CXC^r,PEN^r,AMP^r,AMX^r, AUG^r	0.30	$bla_{AmpC,}bla_{TEM}$, bla_{shv}
	75 (16.1)	ERY^rCOT^r, SXT^r, CHL^r, NIT, TET^r,	0.30	Nil

Table 4: Transformation of R-Plasmid to Recipient *Escherichia coli* K-12

Isolates	Numbers Isolated	Numbers harbouring Plasmid (%)	Numbers resistant after curring (%)	Numbers sensitive after curring (%)	Numbers Conjugated and Transformed to Esch. coli K-12 (mob-Plasmid) (%)	Prevalence of R-Plasmid (%)
Aeromonas hydrphila N=63	63	41 (65.1)	17 (27.0)	44 (69.8)	27 (42.9%)	65.1

Table 2: Percentage resistant and Susceptibility of isolates

Antibiotics, code and concentrations of Oxoid, andAbtek Biologicals England	***Sensitive/Resistance***	***Aeromonas specie (%)***
NIT: Nitrofurantoin 200µg	S I R	356 (76.6) 33 (7.1) 76(16.3)
OFL: Ofloxacin 5µg	S I R	361 (77.6) 77 (16.6) 27 (5.8)
CPR: Ciprofloxacin 5µg	S I R	416(89.5) 32 (6.9) 17 (3.7)
GEN: Gentamicin 30µg	S I R	89 (19.1) 15 (3.2) 361 (77.6)
STR: Streptomycin 30µg	S I R	74 (15.9) 47 (10.1) 344 (74.0)

TET: Tetracycline 30µg	S I R	30 (6.5) 25 (5.4) 411 (88.2)
CHL: Chloramphenicol 30µg	S I R	77 (16.6) 76 (16.3) 312 (67.1)
ERY: Erythromycin10µg	S I R	84 (18.1) 64 (13.8) 317 (68.2)
AUG: Augumentin 30µg	S I R	43 (9.3) 35 (7.5) 387 (83.2)
PEN: Penicillin 30µg	S I R	0 (0.0) 0 (0.0) 465 (100.0)
AMP: Ampicillin 30µg	S I R	5 (1.1) 3 (0.7) 457 (98.3)
CXC: Cloxacillin 30µg	S I R	7(1.5) 10 (2.2) 448 (96.3)
CRX: Cefuroxime 30µg	S I R	178 (38.3) 41 (8.8) 246 (52.9)
CAZ: Ceftazidime 30µg	S I R	79 (17.0) 57 (12.3) 329 (70.8)
CXM: Cefixime 30µg	S I R	78 (16.8) 75 (16.1) 312 (67.1)
CTX: Cefotaxime 30µg	S I R	165 (35.5) 91 (19.6) 209 (45.0)
NAL: Nalidixic acid 30µg	S I R	377 (81.1) 64 (13.8) 24 (5.2)
AMX: Amoxacilin25µg	S I R	9 (1.9) 10 (2.2) 446 (95.9)
SXT: Septrin25µg	S I R	24 (5.2) 18 (3.9) 423 (91.0)

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COT: Cotrimoxazol25µg	S I R	29 (6.2) 33 (7.1) 404 (86.9)
SPC: Spectinomycin 100µg	S I R	28 (6.0) 47 (10.1) 390 (83.9)
MER: Meropenem 10µg	S I R	46 (9.9) 39 (8.4) 380 (81.7)
KAN: Kanamycin 30µg	S I R	33 (7.1) 29 (6.2) 403 (86.7)

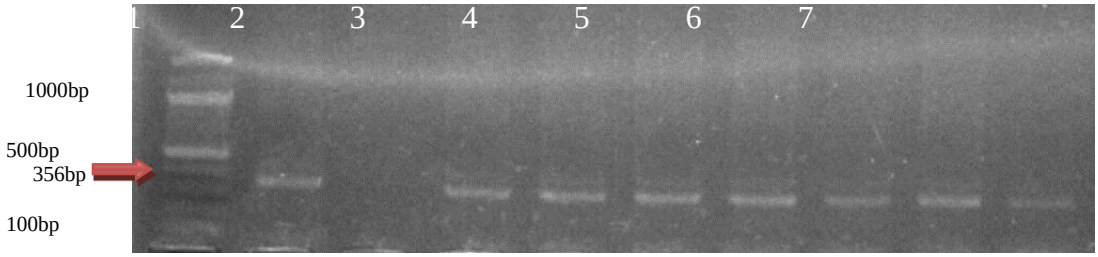

Fig 4: Shows the positive *A. hydrophila* using the 16SrRA gene. L is a Molecular marker while number 1 is a positive control, number 2 (negative control) 3,4,5,6,7,8,9 are positive

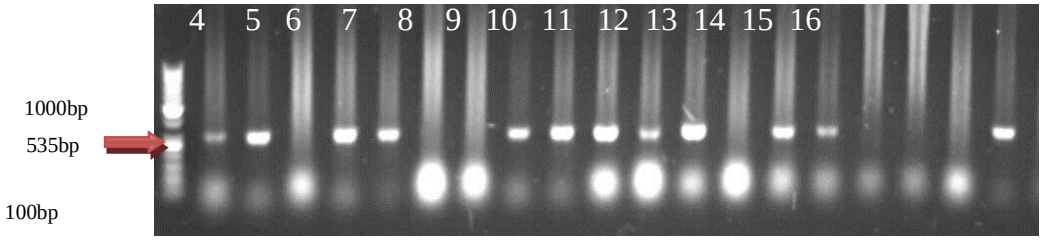

Fig 5: Shows the positive *BlaTem gene*. L is a Molecular marker as number 1 is a positive control, while numbers 2,4,5,8,9,10,11,12,14,15,19 are positive while numbers 3,6,7,13,16, 17,18 are negative

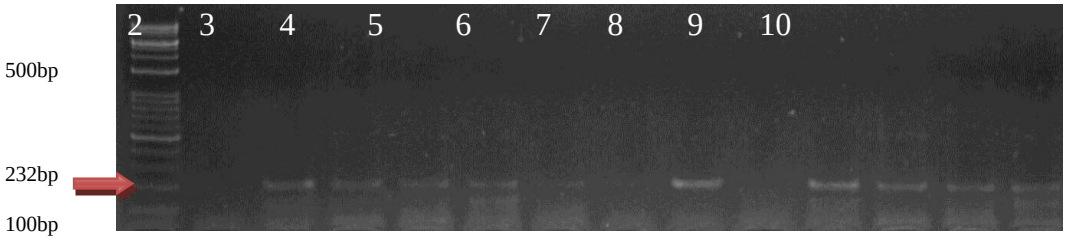

Fig 6: Shows the positive cytotoxic enterotoxin (act) gene. L is a Molecular marker while number 1 is a negative control, number 2 (positive control) 3,4,5,6,7,8,10,11,12,13 are positive samples, while 9 isnegative

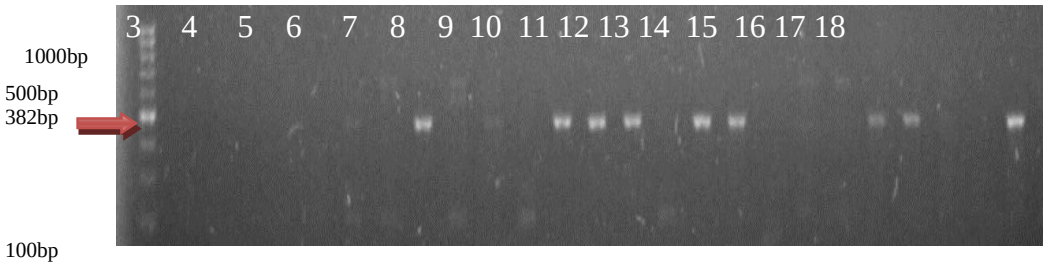

Fig 7: Shows the positive lip gene. L is a Molecular marker while numbers 6,9,10,11,13,14,17,18,21 are positive while numbers 2,3,4,5,7,8,12,15,16,19,20 are negative

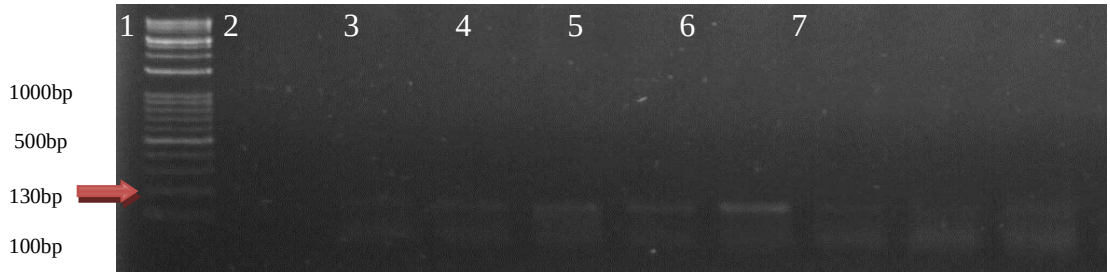

Fig 8: Shows the positive haemolysin (hhly) gene. L is a Molecular marker while Number 1 is a negative control, number 2 (positive control) 3,4,5,6,7,8, 9 are positive

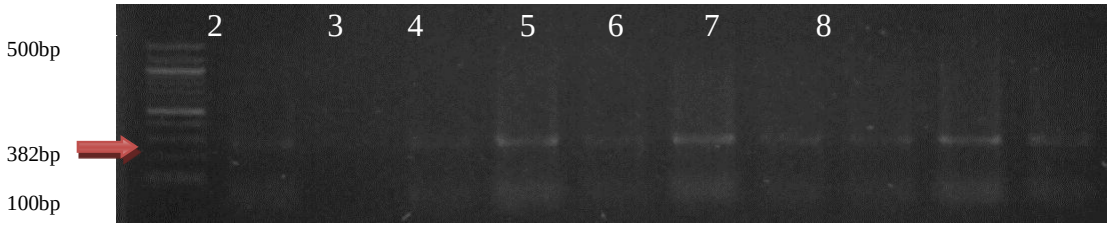

Fig 9: Shows the positive lipase (lip) gene. L is a Molecular marker while Number 1 is a negative control, number 2 (positive control) 3,4,5,6,7,8, 9 are positive

DISCUSSION

Aeromonas species have been implicated in diverse clinical cases affecting man, animals and aquatic lives were its implicated in travellers'diarrhoea, nosocomial infection, and clinical cases associated with hurricanes, tsunamis, and earthquakes (Vila *et al.*, 2003). These cases have shown failure in treatment owning to multiple antibiotic resistances (Igbinosa *et al.*, 2012; Vivas *et al.*, 2004). This study has revealed similar multiple antibiotic-resistant determinants amongst organisms isolated from annelids which probably may not have been exposed to such antibiotics. Amongst the 465 isolates of *Aeromonas* species observed, 63 of them were *A. hydrophila* (fig 4). One of the determinative phenotypic characterizations was amylases production which was indicated by adding 3 ml of Gram's (Lugol's) iodine solution to a starch ampicillin culture media, a yellow to

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slightly brownish colonies were reported as amylase positive colonies. This observation corroborates a previous report by Khamesipour*et al.*,(2014). Amongst the isolated strains, 84 (18.07%) were positive for biofilm formation of which 63 (97.6%) were *A. hydrophila*. Biofilm formation among the pathogen is one of the factors that determine bacterial colonization and attachment to surfaces in their mechanism of infectivity in skin-related infections (carbuncles, furuncle or cellulitis), nosocomial infections, community-acquired infections etc (Vila *et al*., 2003). It's also a factor that enhances pathogens infectivity amongst fish gonads as depicted in the previous report of Guerra *et al.* (2007) that biofilm formation is associated with the pathogenicity of *A. hydrophila* isolates. This finding is in agreement with the recent observation of Igbinosa *et al*. (2015; 2017) and Jiang *et al*. (2015) who reported that biofilm is an important pathogenic factor amongst Aeromonads. Important from our study was the observation of 31/36 (24.6%) strains of *A.hydrophila* isolated from the *H. medicinalis* (Leeches) reporting positive to biofilm formation which is an indication of pathogenic potentials and public health relevance of the isolates. Other health-related genotype observed in the *A.hydrophila* detected were diverse virulent indices which are associated with enterotoxicity, cytotoxicity and lipase. Amongst the total *A.hydrophila* isolated, β-haemolysis was observed in 74.6% (47/63). A similar report was previously reported in Japan, Barcelona and Spain from clinical, food and environmental sources isolates. The studies of Miyagi *et al*. (2016) revealed that 78.3% of *Aeromonas* species isolated from environmental water used for domestic purposes in Okinawa Prefecture, Japan, produces β- haemolysis. This virulent index was further confirmed by the PCR gene-based technique as 74.6% (47/63) were confirmed. Other virulent determinants observed during the study were *Aeromonas cytotoxic enterotoxin* (*act*: 57/63) and lipase (*Lip*: 59/63) as shown in figures 6,7,8,9.

Aeromonads has been reported as an emerging pathogen of high clinical relevance with the increasing acquisition of resistance to numerous antibacterial agents. Multiple Antibiotic Resistance (MAR) is a significant public health concern that has welcome global attention. This study revealed that the *A.hydrophila* isolated possess multiple antibiotic resistance as depicted in Table 3.This result suggests the possible reason for the delay in healing of some patient with infected wound, which were ICU hospitalised in the sampled study area. Consistent observation during the study was a high sensitivity of the organism to members of the fluoroquinolones {Nalidixic acid Ciprofloxacin andOfloxacin}. This result is contrary to a previous report in the United State by Sen and Rodgers, (2004) which suggests that fluoroquinolones have lost their effectiveness due to resistance (Sen and Rodgers 2004; Jalal and Wretlind, 1998), but similar to the reports of Zong *et al.* (2002) that the fluoroquinolone antibiotic members are the most effective against isolated strains. Although *Aeromonads* sensitivity to nitrofurantoin is seldom reported in the literature, it has been seen as an effective antibiotic for the treatment of systemic infections and a possible antibiotic for *Aeromonas* systemic

infection. This study reveals a high susceptibility of the pathogen to nitrofurantoin which is similar to the reports documented by Jombo *et al.* (2008) and Lupiola-Gomez *et al.* (2003). Another important report of the study is the resistant nature of the isolates to the third generation cephalosporin and gentamicin, which are markers of extended-spectrum β-lactamase (*ESβL*). This report is contrary to the previous results from Zong, *et al.* (2002) in the USA that *Aeromonads* are susceptible to third-generation cephalosporin and members of the aminoglycoside. There was also an appreciable resistance to other members of the cephalosporin. This report differs from the observations of Igbinosa *et al*. (2017) on the susceptible nature of the organisms to cefuroxime, although other investigators reports were similar. To affirm the susceptibility or resistance to the third-generation cephalosporins, a culture-dependent method and PCR gene detection were conducted. Result reveals that isolates with positive extended-spectrum β-lactamase markers (*bla*$_{AmpC,}$ *bla*$_{TEM}$, and*bla*$_{shv}$) produce into the medium β-lactamases using the detection method described previously by Igere *et al*. (2020b).The PCR gene detection of such resistant *ESβL* markers further confirmed the phenotypic resistant markers observed. It was observed that73.02% (46/63) were positive to*bla*$_{AmpC,}$ 63.5% (40/63) were positive to *bla*$_{TEM}$, while 46.03% (29/63) of them were positive to*bla*$_{shv}$. All isolates produced PCR amplified products at 535bp, 882bp and 930 bp as reported in the gel documentation system, which affirms the clinical relevance of the pathogens (fig 5).

Our study also reveals that *A.hydrophila* isolated from both *Hirudomedicinalis* and skin swab of users (bathers) of the recreational centres harboured plasmids of similar size (fig 1). A 65.1% (41/63) of the total isolates show plasmid bands with size ranging from 1000bp-20000bp. The isolated plasmids were transferrable from the wide type *A.hydrophila* onto recipient Escherichia coli K-12 strain {33.3%, (21/63)}, indicating that it carries the F-pili or tendency to share extra-chromosomal materials. The 22.2% (14/63) of isolated plasmids which could not be transferred to the standard recipient strains may have resulted from the inability of plasmid reception by recipient strain and the possible rate of plasmid loss during sample analysis. It may also be adjudged as the presence of cryptic plasmid. This is similar to the previous report by Yah *et al*.(2007). Also, 27.0% (17/63) of the multidrug-resistant isolates were chromosomally mediated as the resistance was retained after curing. A similar report was documented in the study of Stratevaand Odeyemi, (2016), who demonstrates the spread of Tet^{r}, plasmids amongst *A. hydrophila* and *E. coli* in men and aquaculture in different geographical regions.

Public health Concern

The isolation of Aeromonads (*Aeromonas hydrophila*) from the gut of leeches (*Hirudomedicinalis*) within Oghara environs indicates that Leeches are potential reservoirs or breading hub of both the potential pathogen and multidrug-resistant strains as the strains has not been implicated in disease case. In addition, the isolates characterized plasmids shows that isolates may have risen from similar origin

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since it has similar size and related resistant markers as those isolated from Leeches (*Hirudomedicinalis*) gut from the recreational centres as depicted in the plasmid DNA analysis. Going by the aforementioned, attention needs to be drawn to this area to prevent resistance spread as *Hirudo medicinalis* has shown a vector tendency for the spread of resistance. Other life's in water bodies such as fishes may also serve as a potential reservoir for the organism and the resistant nature of the organism can result in MAR dissemination to the ecosystem. In the environment, this may affect foodchain, livestock, animal husbandry and human subjects as previously documented (Stratevaand Odeyemi, 2016; Rosenberg-Goldstein *et al*., 2014; Huddleston *et al.*, 2006). There may also be possible potential health concern amongst occupational (fishermen) recreational site visitors who come in contact with water and living organisms (fish etc) and their aerosols downstream regarding *Aeromonads* exposure. This is similar to the previous report of Skwor *et al.* and other investigators that there is a possibility of a contact-borne pathogen spread amongst fish handlers as well as related systemic diseases (Skwor*etal.*,2014; Cabello *et al.*, 2013; Huddleston *et al.*, 2006; Goni-Urriza*et al.*, 2000; Lehane and Rawlin, 2000; Austin and Austin, 1999). It is important to note that succumbing to *A.hydrophila* infections by patients depends on host immune status and some medical conditions hence occupational and recreational exposure is the hot spot that should attract clinicians' interest. The reports of this study are most akin to the reports of Strateva and Odeyemi, (2016).

CONCLUSION

Although leeches used during the study were accessed as apparently healthy, all of them were carrier of the pathogen (*A.hydrophila*),which possibly endanger recreational centre visitors. The phenotypic and biochemical characterization should follow molecular 16SrRNA gene detection and virulence/resistant gene typing. DNA or amplicon sequencing and sequence homology studies were not accessed in the study as funds available could not take care of such. However, the antibacterial resistance profile of isolated *A.hydrophila* and PCR detection of virulent/resistant gene was accessed. The presence of multiple antibiotic-resistant genes and plasmid mediation by the isolates as well as the association of the isolated organisms with plasmid patterns of similar size amongst specimen collected indicates *Hirudo medicinalis* as a breeding hub or reservoir for multiple antibiotic-resistant organism and plasmids. There is a need for a regular and continuous environmental monitoring scheme as well as an increased awareness program on the potential transfer or passage of multiple antibiotic-resistant markers amongst organisms within the Oghara environment.

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Conflict of Interest

The authors declared no conflict of interest

REFERENCES

Adriana Osińska, Monika Harnisz and Ewa Korzeniewska (2016). Prevalence of plasmid-mediated multidrug resistance determinants in fluoroquinolone-resistant bacteria isolated from sewage and surface water. *Environ Sci Pollut Res* 23, 10818–10831.

Ahmed, H.A., Mohamed, M.E., Rezk, M.M., Gharieb, R.M. and Abdel-Maksoud, S.A., (2018). *Aeromonas hydrophila* in Fish and Humans; Prevalence, Virulotyping And Antimicrobial Resistance. Veterinary Medicine In-between Health & Economy (VMHE)–16-19 October 2018, 55(20-Suppl).

Albarral V, A. Sanglas, M. Palau, D. Mi~nana-Galbis, and M.C. Fust_e, (2016). Potential pathogenicity of *Aeromonas hydrophila* complex strains isolated from clinical, food and environmental sources, *Can. J. Microbiol*. **62**, 296-306.

Ali H, F. Miah, S.H. Prodhan, M. Rahman, J. Raihan, K.M.A. Zinnah, T.H. Aunkor, Rahman J, F.B. Ruma and N. Chowdhury, (2014). Putative virulence and antimicrobial susceptibility of *Aeromonas* spp. isolated from marketed fish intended for human consumption in Bangladesh, *Asian J. Microbiol. Biotech. Env*. **16**, 485-495.

Amita Jain and Rajesh Mondal, (2008). TEM & SHV genes in extended-spectrum β-lactamase producing *Klebsiella* species & their antimicrobial resistance pattern. *Indian J Med Res*. **128**, pp 759-764.

Austin,B., and Austin,D, (1999). In: Laird,L.(ed.), Bacterial fish pathogens: disease in farmed and wild fish, 3rd ed. Springer-Praxisseries in aquaculture and fisheries, 455pp.

Awan, B., Maqbool, A., Bari, A. and Krovacek, K. (2009). Antibiotic susceptibility profile of *Aeromonas* spp. isolates from food in Abu Dhabi, United Arab Emirates. *New Microbiologica*. 32(1), 17–23.

Boris, S. and Peter, T. (2008). "Global diversity of leeches (Hirudinea) in freshwater". Freshwater Animal Diversity Assessment. *Hydrobiologia*. 595**,** pp. 129–137.

Bosch, T., Schade, R., Landman, F., Schouls, L. and Dijk, K.V. (2019). A bla VIM-1 positive *Aeromonas hydrophila* strain in a near-drowning patient: evidence for interspecies plasmid transfer within the patient. *Future Microbiology*, **14**(14), pp.1191-1197.

Busing, K.H., W. Doll and K. Freytag, (1953). Die baklerien, flore der medizinischenblultegel. *Arch. Mikrobiol*. 19, 52-86.

Cabello, F.C., Godfrey,H.P., Tomova,A., Ivanova,L., Dolz,H., Millanao,A., and Busch- Mann, A.H., (2013). Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and animal and human health. *Environ. Micro-boil*. 15(7), 1917–1942.

CLSI. *Clinical and Laboratory Standards Institute.* (2015) *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Information Supplement*; M100-S25; Clinical and Laboratory Standards Institute: Wayne, PA, USA.

Daskalov, H. (2006). The importance of *Aeromonas hydrophila* in food safety. *Food Control.*17(6), 474–483.

Deyan Strateva and Olumide A. Odeyemi, (2016). Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review. *Journal of Infection and Public Health*. **9**, 535—544.

Dos Santos Silva, A., Soares, L.S., das Virgens Lima, D. and Velame, D.S.

Scientia Africana, Vol. 20 (No. 2), August, 2021. Pp 145-166 *https://dx.doi.org/10.4314/sa.v20i2.13*
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(2019). *Aeromonas* spp in fish and in continental waters. *Revista Brasileira de Higiene e Sanidade Animal: RBHSA*, 13(1), pp.15-47.

El-ghareeb, H.M., Zahran, E. and Abd-Elghany, S.M., 2019. Occurrence, Molecular Characterization and Antimicrobial Resistance of Pathogenic *Aeromonas hydrophila* from Retail Fish. Alexandria Journal for Veterinary Sciences, 62(1).

Emmanuel E. Odjadjare, Etinosa O. Igbinosa, Raphael Mordi, Bright Igere, Clara L. Igeleke and Anthony I. Okoh, (2012). Prevalence of Multiple Antibiotics Resistant (MAR) *Pseudomonas* Species in the Final Effluents of Three Municipal Wastewater Treatment Facilities in South Africa. *Int. J. Environ. Res. Public Health* **9**, 2092-2107; doi:10.3390/ijerph 9062092.

Enany M., Ibrahim H., Atta A.M., Adawy M.M. (2011). Bacteriological and histopathological studies on some bacterial pathogens causing diseases in cultured Mugil Capito fish in Ismailia Governorate, *SCVMJ* 16, 1-9

Faham Khamesipour, Mitra Moradi, Esmat Noshadi and Manouchehr Momeni Shahraki,(2014). Detection of the prevalence of *Aeromonas hyrophila* in shrimp samples by polymerase chain reaction (PCR) and cultural method in Iran. *Journal of Biodiversity and Environmental Sciences (JBES)*. **4(**2), 47-52.

Goñi-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, P. and Quentin C. (2000a). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* spp**.** *Applied and Environmental Microbiology***.** 66(1), 125–132

Goñi-Urriza, M., Pineau, L., Capdepuy, M., Roques, C., Caumette, P. and Quentin, C. (2000b). Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. *Journal of Antimicrobial Chemotherapy*.46(2), 297–301.

Guerra I.M.F, Fadanelli R., Figueiro M., Schreiner F., Delamare A.P.L., Wollheim C., *et al.*. (2007). *Aeromonas* associated diarrhoeal disease in South Brazil: prevalence, virulence factors and antimicrobial resistance, *Braz. J. Microbiol*. 38, 638-643.

Hafez, A.E.E., Darwish, W.S., Elbayomi, R.M., Hussein, M.A. and El Nahal, S.M. (2018). Prevalence, Antibiogram And Molecular Characterization of *Aeromonas hydrophila* Isolated From Frozen Fish Marketed in Egypt. *Veterinary Medicine In-between Health & Economy (VMHE)*. 16-19 October 2018, 55(20-Suppl).

Huddleston, J.R., Zak,J.C., and Jeter,R.M, (2006). Antimicrobial susceptibilities of *Aeromonas* spp. Isolated from environmental sources. *Appl.Environ. Microbiol*.72 (11), 7036–7042.

Huys, G. and Cnockaert, M. (2005). *Aeromonas culicicola* is a later subjective synonym of *Aeromonas veronii Syst. Appl. Microbiol*. 28, 604–609.

Igbinosa E.O and E.E. Odjadjare, in: A. M_endez-Vilas (Ed.), (2015a). Antibiotics and Antibiotic Resistance Determinants: an Undesired Element in the Environment, the Battle against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs, vol. 2, Formatex Research Center, Extremadura, Spain, pp. 858-866.

Igbinosa I.H, E.U. Igumbor, F. Aghdasi, M. Tom and A.I. Okoh, (2012). Emerging *Aeromonas* species infections and their significance in public health, *Sci World J*, http://dx.doi.org/10.1100/2012/ 625023. Article ID 625023, 13 pages.

Igbinosa I.H., A. Beshiru and E.O. Igbinosa, (2017). Antibiotic resistance

profile of *Pseudomonas aeruginosa* isolated from aquaculture and abattoir environments in urban communities, *Asian Pac. J. Trop. Dis*. 7, 930-935.

Igbinosa I.H., E.O. Igbinosa, and A.I. Okoh, (2015b). Detection of antibiotic resistance, virulence gene determinants and biofilm formation in *Aeromonas* species isolated from cattle, *Environ. Sci. Pollut. Res.***22**, 17596-17605.

Igere BE, Ekundayo TC, (2020a). Global mapping of cholera *Vibrio* and outbreaksin the Pre-Millennium Development Goals (MDG)/ Sustainable Development Goals (SDG) and MDGs/ SDGs era of 1990–2019, *Microbial Pathogenesis*, doi: https://doi.org/10.1016/j.micpath.2020.104319.

Igere BE, Okoh AI, and Nwodo UU. (2020b). Antibiotic Susceptibility testing reports: A basis for environmental/ epidemiological surveillance and infection control amongst environmental Vibrio cholerae. *International J. of Environ Res and Pub Health*17;121.

Jalal, S. and Wretlind, B. (1998). Mechanisms of quinolone resistance in clinical strains of *Pseudomonas aeruginosa*. *Microb. Drug Resist*.4, 257–261.

Janda, J.M. and Abbott S.L. (2010). The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews. 23(1), 35–73.

Jombo GT, Jonah P, and Ayeni JA, (2008). Multidrug-resistant *Pseudomonas aeruginosa* in contemporary medical practice: findings from urinary isolates at a Nigerian university teaching hospital. *Nig. J. Physiological Sciences*. 23, 105-109.

Khajanchi B.K, Fadl A.A., Borchardt M.A., Berg R.L., Horneman A.J., Stemper M.E., Joseph S.W., Moyer N.P., Sha J. and Chopra A.K. (2010). Distribution of virulence factors and molecular fingerprinting of *Aeromonas* species isolates from water and clinical samples: suggestive evidence of water-to-human transmission. *App. Environ. Microbiol*. 76, 2313-2325.

Khajanchi B.K, Sha J., Kozlova E.V., Erova T.E., Suarez G., Sierra J.C., Popov V.L., Horneman A.J., and Chopra A.K. (2009). N-Acylhomoserine lactones involved in quorum sensing control the type VI secretion system, biofilm formation, protease production, and in vivo virulence in a clinical isolate of *Aeromonas hydrophila*, *Microbiol*. **155,** 3518-3531.

Korkoca H, Y. Alan, S. Bozarı, M. Berktas¸ and Y. Goz, (2014). Detection of putative virulence genes in *Aeromonas* isolates from humans and animals. *J. Infect. Dev. Ctries*. **8,** 1398-1406.

Lehane, L., and Rawlin,G.T, 2000. Topically acquired bacterial zoonoses from fish: a review. *Med.J.Aust*. 173(5), 256–259.

Lupiola-Gomez, P., Gonzalez-Lama, Z., Tejedor-Junco, M., Gonzalez-Martin, M. and Martin-Barrasa, L. (2003). Group 1 beta-lactamases of *Aeromonas caviae* and their resistance to beta-lactam antibiotics. *Canadian Journal of Microbiology*. 49(1), 207–215.

Miyagi K, Hirai I. and Sano K. (2016). Distribution of *Aeromonas* species in environmental water used in daily life in Okinawa Prefecture, *Japan, Environ. Health Prev. Med*. 21, 287-294.

Mudaliar, N., Kanojia, N., Shaikh, K. and Rathod, S. (2019). Detection of Drug-Resistant Organisms From Natural Water Bodies. *Journal of applied microbiology*.

Nawaz M, Khan SA, Khan AA, Sung K, Tran Q, Kerdahi K, Steele R. (2010). Detection and characterization of

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virulence genes and integrons in *Aeromonas veronii*isolated from catfish. *Food Microbiol* 27 (3), 327–31.

Osińska, Adriana, Monika Harnisz, and Ewa Korzeniewska. (2016). "Prevalence of plasmid-mediated multidrug resistance determinants in fluoroquinolone-resistant bacteria isolated from sewage and surface water", *Environmental Science and Pollution Research*.

Pidiyar, V., Kaznowski, A., Badri Narayan, N., Patole, M. and Shouche Y. (2002). *Aeromonas culicicola* sp. nov., from the midgut of *Culex quinquefasciatus*. *International Journal of Systematic and Evolutionary Microbiology*. 52(5), 1723–1728.

Pougnet, R., Pougnet, L., Allio, I., Lucas, D., Dewitte, J.D. and Loddé, B. (2018). Maritime environment health risks related to pathogenic microorganisms in seawater. *International maritime health*, 69(1), pp.35-45.

Rosenberg Goldstein, R., Micallef, S.A., Gibbs, S.G., George, A., Claye, E., Sapkota, A., Joseph, S.W. and Sapkota, A.R, (2014). Detection of vancomycin-resistant enterococci (VRE) at four U.S. wastewater treatment plants that provide effluent for reuse. *Sci. Total Environ*. 466–467, 404–411.

Sambrook J., Fritshch E.F. and Maniatis T. (1989). Molecular Cloning a Laboratory Manual 2nd edition. Cold Spring Harbour Laboratory Press, USA. 22.104p

Schuetz, A.N., (2019). Emerging agents of gastroenteritis: *Aeromonas*, *Plesiomonas*, and the diarrheagenic pathotypes of *Escherichia coli*. In Seminars in diagnostic pathology. WB Saunders.

Scott A. Forrester. (2014). The benefit of Campus recreation. NIRSA Headquarters 4185 SW Research Way Corvallis, Oregon 97333-1067. Pg 1-26. www.nirsa.org.

Sen, K. and Rodgers, M. (2004). Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. *Journal of Applied Microbiology*. 97(5), 1077–1086.

Shah D, Shringi S, Besser T. and Call D (2009). Molecular detection of foodborne pathogens. Boca Raton: CRC Press, In Liu, D. (Ed). Taylor &Francis Group, Florida, USA Pp. 369–89.

Sharma, S.; Sachdeva, P. and Virdi, J.S, (2003). Emerging water-borne pathogens. *Appl. Microbiol. Biotechnol*. 61, 424–428.

Skwor, T., Shinko,J., Augustyniak,A., Gee,C., and Anrdraso,G, (2014). *Aeromonas hydrophila* and *Aeromonas veronii* predominate among potentially pathogenic ciprofloxacin-and tetracycline-resistant Aeromonas isolates from Lake Eerie. *Appl. Environ. Microbiol*. 80(3), 841–848.

Stratev D, Gurova E, Vashin I. and Daskalov H, (2016). Multiplex PCR detection of haemolysin genes in β-haemolytic *Aeromonas hydrophila* strains isolated from fish and fish products. *Bulgarian J Agricul Sci*. 22, 308–14.

United States Environmental Protection Agency. (2001). *Method of 1605. Aeromonas in Finished Water by Membrane filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)*Washington, DC, USA: United States Environmental Protection Agency.

Vermont, (2012). Healthy Recreational Waters.Vermont Department of Health/Healthy Recreational Waters at 1-800-439-8550. Option 6 (VT only) or 1-802-863-7220 or vdh.environmentalhealth@state.vt.us.

Vila, J., Marco, F., Soler, L., Chacon M. and Figueras, J. (2002). In vitro antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas veronii* biotype *sobria*. *Journal of Antimicrobial Chemotherapy*. 49(4), 701–702.

Vivas, J., Carracedo B, and Riaño J, (2004). Behaviour of an *Aeromonas hydrophilaaroA* live vaccine in water microcosms. *Applied and Environmental Microbiology*. 70(5), 2702–2708.

Wang G, Clark C, Liu C, Pucknell K, Munro C, Kruk T, Caldeira R, Woodward D and Rodgers F. (2003). Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. *J Clin Microbiol.* 41 (3): 1048–54.

WHO, (2003). Guidelines for Safe Recreational Water Environments. *volume 1: coastal and freshwaters.* world health organization, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. *Pg 1-253.*

Wroblewska M.M, Dijkshoorn L. and Marchel H. (2004). Outbreak of nosocomial meningitis caused by *Acinetobacter baumannii* in neurosurgical patients. *J. Hosp. Infect.* **57,** 300-307.

X. Jiang, Y.X. Qin, G.F. Lin, L. Huang, B. Huang, W.S. Huang and Q.P. Yan, (2015).FlgN plays important roles in the adhesion of *Aeromonas hydrophila*to host mucus. *Genetics and Molecular Research*. **14** (2), 6376-6386.

Yah S.C., Eghafona N.O., Oranusi S. and Abouo A.M (2007). Widespread Plasmid Resistance genes among Proteus species in diabetic Wounds of Patients in the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. African Journal of Biotechnology.**6**(15), 1757-1762.

Yang, Y. and Livemore, D.M. (1989). Interactions of FCE 22101 with class 1 β-Lactamase. *J. Antimicrob Chemother*.**23**, Suppl. C: 85 – 94.

Zong, Z., Lu, X. and Gao, Y. (2002). *Aeromonas hydrophila* infection: clinical aspects and therapeutic options. *Reviews in Medical Microbiology*.13(4), 151–162.