Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Microbiological research https://doi.org/10.12980/apjtd.7.2017D6-363 ©2017 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Antibiotic resistance profile of *Pseudomonas aeruginosa* isolated from aquaculture and abattoir environments in urban communities

Isoken Henrietta Igbinosa[1,2], Abeni Beshiru[3], Etinosa Ogbomoede Igbinosa[1,3*]

[1]*SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Private Bag X1314, Alice, 5700, South Africa*

[2]*Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria*

[3]*Applied Microbial Processes and Environmental Health Research Group, Department of Microbiology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria*

ARTICLE INFO

Article history:
Received 10 Oct 2016
Received in revised form 21 Oct 2016
Accepted 31 Oct 2016
Available online 18 Nov 2016

Keywords:
Multidrug resistance
Pathogen
Health risk
Effluent
Public health

ABSTRACT

Objective: To characterize multiple antibiotic resistance profile of *Pseudomonas aeruginosa* from aquaculture and abattoir environments.

Methods: Wastewater samples were obtained from the abattoir and aquaculture environments between May 2016 and July 2016 and analysed using standard phenotypic, biochemical and PCR-based methods.

Results: The mean pseudomonads count ranged from ($4 \times 10^2 \pm 1.01$) to ($2 \times 10^4 \pm 0.10$) colony-forming unit/mL in the aquaculture environment and ($3 \times 10^3 \pm 0.00$) to ($1 \times 10^5 \pm 1.00$) colony-forming unit/mL in the abattoir environment. A total of 96 isolates of *Pseudomonas aeruginosa* confirmed by PCR were thereafter selected from both aquaculture and abattoir environments and further characterized for their antimicrobial susceptibility profile by adopting the disc diffusion method. High level of resistance was observed against the aminoglycosides [gentamycin 64/96 (66.67%) and kanamycin 52/96 (54.17%)], monobactams [aztreonam 76/96 (79.17%)], carbapenems [meropenem 52/96 (54.17%)], tetracyclines [tetracycline 72/96 (75.00%)] and cephems [ceftazidime 72/96 (75.00%) and cefuroxime 48/96 (50.00%)]. Multiple antibiotic resistant index of the respective isolates ranged from 0.4 to 0.8 while multidrug resistant profile of the isolates revealed that 28 of the respective isolates were resistant to ceftazidime, cefuroxime, gentamycin, kanamycin, aztreonam which belongs to cephems, aminoglycosides and monobactam class of antimicrobials.

Conclusions: Findings from the present study therefore underscores the need for effective monitoring of the abattoir and aquaculture environments as they could be the significant source for spreading antibiotic resistant bacteria within the environment.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative, oxidase positive organism found in various ecological niches including soil, water, rhizosphere, plant, animal and sewage environment[1,2]. It comprises strains with economic, ecological and health-related significance and has also been categorized as an emerging waterborne pathogen[3,4]. Species of *Pseudomonas* have been implicated in infections of public health importance among which include cystic fibrosis, urinary tract infections, bone and joint, gastrointestinal infection, bacteremia, soft tissue and respiratory system. They are also of concern in immune-compromised patients such as AIDS and cancer infected patients[5]. *Pseudomonas* as an emerging pathogen is centred principally within their capability to exist in biofilms (a combined population of bacterial that adheres to specialized surfaces within the aquatic ecosystem) that frequently serves as shielding asylum for the bacteria against physical, biological, environmental and chemical stress conditions. Proliferation inside biofilms enhances genetic diversity which, in turn, heightens the potential for susceptibility against antimicrobials, disinfectants, environmental stress as well as increases the virulence capacity of the pathogen[6].

Wastewater effluents from abattoir environments are usually

*Corresponding author: Etinosa Ogbomoede Igbinosa, SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Private Bag X1314, Alice, 5700, South Africa.

Tels: +27 (0) 83 246 7855 (IH Igbinosa); +27(0) 84 366 6681 (EO Igbinosa)

E-mails: ladyisoken@gmail.com (IH Igbinosa); eigbinosa@gmail.com (EO Igbinosa)

Foundation Project: Supported by The World Academy of Science, Italy (Grant No. 14-091 RG/BIO/AF/AC_1-UNESCO FR: 324028575).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

characterized by the presence of pathogenic microorganisms which include the genus *Pseudomonas*, *Aeromonas* and *Enterococcus* species which are of significance to public health[3,7]. The presence of these organisms coupled with their inherent capacity to resist multiple antibiotics is an enigmatic problem of public health importance[5]. These multidrug resistant pathogenic bacterial isolates which might have emanated from the carcases as a consequence of the continuous use and misuse of antimicrobials as therapeutic/prophylactic agent as well as growth promoters can be transferred via the food chain to humans[8,9]. Resistance to antimicrobials by pathogenic bacterial isolates can also be a consequence of the ability of the organisms to harbour resistant genes and transmit these genes within bacterial populations via horizontal gene transfer and mobile genetic elements (transposons, plasmids, *etc.*)[10,11]. This is a crucial issue as these organisms might migrate into the food chain resulting in food infection or directly to humans resulting in difficulties to treat infections[12].

Microbial drug resistance is a significant public health concern. There has been less attention paid to the potential for antibiotic use in the aquaculture and abattoir environments, which compromises environmental and human health. In developed countries, such as members of the European Union, Great Britain, United States of America, Canada and Norway, the use of antibiotics (as growth promoters, prophylactic/therapeutic agents) is declining because of the progresses in management and advances of effective vaccines[13]. However, this does not seem to be followed suit in Nigeria where monitoring controls are fragile and compromised, and the administration of antibiotics seems to be abused.

Previous studies have isolated and characterized *Pseudomonas* species from poultry[14], abattoir environments[1,3,4,7,15], effluent water from a brewery[16] and fresh and dry fish species[12,17]. Abattoirs, aquaculture environment and associated wastewaters are potential sources of pathogenic bacteria that could possess, act as reservoirs and spread antibiotic resistance genes within bacterial population to food of animals as well as humans. Thus, the present investigation aimed to detect and characterize the multiple antimicrobial susceptibility profile of *P. aeruginosa* from aquaculture and abattoir environments.

2. Materials and methods

2.1. Study area

The site of the sampling fish ponds (aquaculture) was located at Ikpoba-Okha and Egor Local Government Areas (LGA) of Benin Metropolis, Edo State, Nigeria. The pond at Ikpoba-Okha LGA was a trampoline pond. Respective ponds examined were periodically drained on a daily basis. Fishes were fed in the morning and evening with commercially available feed. No antibiotic was given to the fish or placed in the water except in cases where there were challenges like spotting on the fish in the ponds. More so, fowl droppings were placed in the pond at Ikpoba-Okha LGA for a period of 3 days before the fishes were placed in the pond at the fingerling stage. The pond at Egor LGA was a concrete pond. Artificial fertilizer was also used to facilitate the growth of green algae in the fish pond. No animal waste was used in fish ponds from Egor LGA.

Slaughter houses (abattoir) investigated were located in Ikpoba-Okha LGA. Cattles were slaughtered on a daily basis, chopped to sizes and transported to different open markets and cold rooms where they were sold out to consumers. The abattoir in this LGA was located close to the Ikpoba River. As such, all wastewaters from the abattoir found their way directly to the river or indirectly to the river through leaching or erosion.

2.2. Sample collection

Wastewater samples were collected on a bimonthly basis at margins and in the middle of the pond in 50 mL sterile universal containers. Wastewater samples were collected from different points at the abattoir such as the drainage, stagnant water around the abattoir environment and washed meat slaughtered at the abattoir using 50 mL sterile universal containers and were labelled appropriately. Samples were collected between May and July, 2016. All samples were transported in cold ice pack to the Applied Microbial Processes & Environmental Health Research Group Laboratory, Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, where they were analysed within 4 h after collection.

2.3. Enumeration of Pseudomonas

All samples were serially diluted to obtain dilution factors (10^{-1} –10^{-3}). An aliquot of 100 μL was then transferred from each of the diluents to glutamate starch phenol red agar (Merck, Germany) and cetrimide agar (Merck, Germany) in triplicates with the aid of sterile pipette and spread on the solidified agar surface by spreading the aliquot with a sterile glass rod. The plates were allowed to stand for 10–20 min before they were incubated at (37 ± 2) ℃ for 18 h. After incubation, discrete colonies (pink colonies) and fluorescent, green colonies were counted and expressed as colony-forming unit (CFU)/mL.

2.4. Enrichment and isolation method

An aliquot of 100 μL from each of the samples obtained was dispensed into a conical flask containing 5 mL of tryptone soy broth (Merck, Germany) and incubated at room temperature of (37 ± 2) ℃ for 24 h. Thereafter, streak plate method was employed in streaking the 18–24 h old broth medium on Glutamate Starch Phenol Red Agar which was selective for the isolation of *Pseudomonas* and *Aeromonas* species and incubated for 18 h at (37 ± 2) ℃. After incubation, discrete colonies (pink colonies) were sub-cultured on cetrimide agar for another 18 h to obtain pure *Pseudomonas* isolates. Gram staining was carried out on isolates. All *Pseudomonas* isolates were stored at –20 ℃ in brain heart infusion broth with 30% glycerol.

2.5. Biochemical characterization of the isolates

Gram-negative rod shapes and polar flagellated oxidase positive isolates were biochemically characterized using the Analytical

Profile Index 20NE according to the manufacturer's instructions (BioMerieux, Marcy-l'Étoile, France) as previously described by Igbinosa *et al.*[3]. Positive control used included *P. aeruginosa* (ATCC 27853). The strips were studied and final identity of the isolates was revealed adopting Analytical Profile Index lab plus software (BioMerieux, Marcy l'Etoile, France).

2.6. Extraction of genomic DNA

Discrete colonies of *P. aeruginosa* cultivated at 37 ˚C on tryptone soy agar plates were selected and re-suspended on 100 µL of sterilized distilled water. The presumptive cells were lysed with the heat block (MK200-2, Shanghai, China) at 100 ˚C for 15 min. The preparation was thereafter centrifuged at 11 000 r/min for 2 min using a MiniSpin microcentrifuge to remove the cell debris. The supernatant was then stored at –20 ˚C and used as DNA template.

2.7. PCR amplification assay

Presumptive *P. aeruginosa* isolates previously characterized using phenotypic and biochemical methods were further confirmed using PCR. All PCR amplification procedures were accomplished in 22.5 µL volume of reaction buffer which contained 2.5 µL of DNA template and 0.05 IU/mL *Taq* polymerase recommended by the manufacturer (Fermentas Life Sciences, USA). DNA templates of *P. aeruginosa* ATCC 27853 was used as a positive control while sterilized distilled H_2O was added as a negative control into each PCR assay. All PCR assay was carried out using a Peltier-based thermal cycler (Bio-Separation System, Shanxi, China) at the succeeding thermal cycler conditions: 95 ˚C for 1 min; 40 rounds of thermal denaturation at 95 ˚C for 15 s; primer annealing at 58 ˚C for 20 s; concluding extension at 68 ˚C for 40 s and holding temperature of 4 ˚C. *P. aeruginosa* primers used were pa722F (5'-GGCGTGGGTGTGGAAGTC-3') and pa899R (5'-TGGTGGCGATCT TGAACT TCTT-3') amplicon size of 199 bp[18]. Amplicons were electrophoresed with 1% agarose gel (Hispanagar, Spain) encompassing ethidium bromide 0.5 mg/L (Merck, SA) at 100 V for 1 h in 0.5 × tris-acetate-ethylene diamine tetraacetic acid buffer (40 mmol/L tris-HCl, 20 mmol/L Na-acetate, 1 mmol/L ethylene diamine tetraacetic acid, pH 8.5) and imagined under a UV transilluminator (EBOX VX5, Vilber Lourmat, France).

2.8. Antimicrobial susceptibility testing

Susceptibility profiles of the *P. aeruginosa* isolates were determined by the disc diffusion technique of Kirby Bauer on Mueller-Hinton agar (Merck, Germany) plates. For the disc diffusion assay, bacteria were cultivated between 18 and 24 h on Mueller-Hinton agar, reaped and then re-suspended in 0.85% sterilized physiological saline solution that was adjusted to a 0.5 McFarland turbidity standard, conforming to 10^8 CFU/mL. The inoculum was thereafter streaked onto plates of Mueller-Hinton agar with the aid of a sterilized cotton swab and aseptically impregnated with commercially available antibiotics discs acquired from Mast Diagnostics, Merseyside, United Kingdom. The *P. aeruginosa* isolates were then subjected to 10 antibiotics belonging to 7 groups of antimicrobials. The antibiotics used were penicillins [piperacillin (100 µg)], cephems [ceftazidine (3 µg), cefuroxime (30 µg)], monobactams [aztreonam (30 µg)], carbapenems [ertapenem (10 µg), meropenem (10 µg)], aminoglycosides [gentamicin (10 µg), kanamycin (30 µg)], tetracyclines [tetracycline (30 µg)] and fluoroquinolones [ciprofloxacin (5 µg)]. After incubated for 24 h at room temperature, organisms were classified as sensitive (S) or resistant (R) on the basis of the clear zone of inhibition in accordance to the guidelines of the Clinical and Laboratory Standard Institute[19].

2.9. Statistical analysis

All data were analyzed using the statistical package IBM SPSS version 21.0. Descriptive statistics, one-sample *t*-test and One-way ANOVA were adopted. $P < 0.05$ was considered statistically significant.

3. Results

The mean *P. aeruginosa* counts from aquacultures and abattoir were presented. During the month of May, June and July, the mean *Pseudomonas* counts from aquacultures in Ikpoba-Okha were ($2 \times 10^3 \pm 0.96$), ($2 \times 10^4 \pm 0.07$) and ($2 \times 10^4 \pm 0.10$) CFU/mL, respectively, while those of Egor were ($4 \times 10^2 \pm 1.01$), ($5 \times 10^2 \pm 0.11$) and ($3 \times 10^3 \pm 0.01$) CFU/mL, respectively ($P < 0.05$). During the month of May, the mean *P. aeruginosa* counts from drains, environment and wash water in the abattoir were ($4 \times 10^3 \pm 0.10$), ($1 \times 10^5 \pm 1.00$) and ($2 \times 10^4 \pm 0.01$) CFU/mL, respectively ($P < 0.05$). In the month of June, the mean counts were ($3 \times 10^3 \pm 0.00$), ($9 \times 10^4 \pm 0.00$) and ($1.2 \times 10^4 \pm 0.00$) CFU/mL, respectively, while in July, the mean counts were ($1 \times 10^4 \pm 0.01$), ($7 \times 10^4 \pm 0.00$) and ($7 \times 10^3 \pm 0.10$) CFU/mL, respectively ($P < 0.05$).

The resistance profile of the 96 *P. aeruginosa* detected from abattoir and fish pond water was further phenotypically characterized using ten antibiotics which were representatives of seven groups of antimicrobial agents. The resistance profile of the isolates revealed that 12/96 (12.50%) of the isolates were resistant to piperacillin, 72/96 (75.00%) were resistant to ceftazidime and tetracycline, 48/96 (50.00%) were resistant to cefuroxime, 64/96 (66.67%) were resistant to gentamycin, 52/96 (54.17%) were resistant to kanamycin, 8/96 (8.33%) were resistant to ertapenem, 16/96 (16.67%) were resistant to ciprofloxacin, 52/96 (54.17%) were resistant to meropenem and 76/96 (79.17%) of the isolates were resistant to aztreonam (Table 1).

The antimicrobial susceptibility profile of the *P. aeruginosa* isolates from aquacultures showed that none of the isolates were resistant to cefuroxime, ertapenem and ciprofloxacin in Ikpoba-Okha and Egor LGAs while they revealed different levels of resistance to other antibiotics assayed. More so, all isolates from abattoir were sensitive to piperacillin and resistant to other antibiotics used in variable percentages of resistance (Table 2). A clear pattern of multiple antibiotic resistance (MAR) along the lines of some *P. aeruginosa* isolates origin was observed in the present study. The MAR index ranged from 0.4 to 0.8. The modal value of MAR index

Table 1
Antimicrobial agents, zone diameter interpretative standards, disc content and resistance profile of *P. aeruginosa* from aquaculture and abattoir.

Antibiotics group	Antimicrobial agent	Disc content (μg)	Zone diameter (mm)			Resistant *P. aeruginosa* (%)
			Resistant	Intermediate	Sensitive	
Penicillins	Piperacillin	100	⩽ 14	15–20	⩾ 21	12/96 (12.50)
Cephems	Ceftazidime	30	⩽ 14	15–17	⩾ 18	72/96 (75.00)
	Cefuroxime	30	⩽ 20	17–19	⩾ 16	48/96 (50.00)
Aminoglycosides	Gentamycin	10	⩽ 12	13–14	⩾ 15	64/96 (66.67)
	Kanamycin	30	⩽ 13	14–17	⩾ 18	52/96 (54.17)
Monobactams	Aztreonam	30	⩽ 15	16–21	⩾ 22	76/96 (79.17)
Carbapenems	Ertapenem	10	⩽ 18	19–21	⩾ 22	8/96 (8.33)
	Meropenem	10	⩽ 15	16–18	⩾ 19	52/96 (54.17)
Fluroquinolones	Ciprofloxacin	5	⩽ 15	16–20	⩾ 21	16/96 (16.67)
Tetracyclines	Tetracycline	30	⩽ 11	12–14	⩾ 15	72/96 (75.00)

for the isolates was 0.6. The least pattern of MAR was resistant to 4/10 antibiotics while the highest level of MAR was resistant to 8/10 antibiotics (Table 3). Extensive drug resistance profile in the present study showed that 36 *P. aeruginosa* were resistant to ceftazidime, cefuroxime, gentamycin and kanamycin while multidrug resistant (MDR) profile in the present study showed that 28 *P. aeruginosa* were resistant to ceftazidime, cefuroxime, gentamycin, kanamycin and aztreonam (Table 4).

Table 2
Distribution of the resistant profile of *P. aeruginosa* from abattoir and aquaculture.

Antimicrobial agents	Abattoir [*n* (%)]			Aquaculture [*n* (%)]		*P*
	Drains (*n* = 23) (%)	Environment (*n* = 16)	Wash water (*n* = 9)	Ikpoba-Okha (*n* = 19)	Egor (*n* = 29)	
Piperacillin	0 (0.00)	0 (0.00)	0 (0.00)	3 (15.78)	10 (34.48)	0.001
Ceftazidime	23 (100.00)	16 (100.00)	9 (100.00)	9 (47.37)	15 (51.72)	0.019
Cefuroxime	23 (100.00)	16 (100.00)	9 (100.00)	0 (0.00)	0 (0.00)	0.001
Gentamycin	23 (100.00)	16 (100.00)	4 (44.44)	7 (36.84)	19 (65.52)	0.010
Kanamycin	23 (100.00)	16 (100.00)	5 (55.56)	7 (36.84)	5 (17.24)	0.017
Aztreonam	23 (100.00)	8 (50.00)	9 (100.00)	10 (52.63)	29 (100.00)	0.020
Ertapenem	0 (0.00)	9 (56.25)	0 (0.00)	0 (0.00)	0 (0.00)	0.051
Meropenem	12 (52.17)	10 (62.50)	0 (0.00)	16 (84.21)	19 (65.51)	0.000
Ciprofloxacin	12 (52.17)	7 (43.75)	0 (0.00)	0 (0.00)	0 (0.00)	0.040
Tetracycline	23 (100.00)	16 (100.00)	9 (100.00)	10 (52.63)	15 (51.72)	0.034

Table 3
MAR index of *P. aeruginosa* isolates from abattoir and aquaculture.

Isolate code	Antibiotics	MAR index
PDR1	PTZ, CAZ, GEN, KAN, ATM, MEM, TET	0.7
PDR2	CAZ, CXM, KAN, ATM, ETP, MEM, TET	0.7
PDR3	CAZ, CXM, GEN, KAN, ATM, MEM, CIP, TET	0.8
PDR4	CAZ, CXM, GEN, KAN, ETP, MEM, CIP, TET	0.8
PEN1	PTZ, CAZ, GEN, KAN, ATM, MEM	0.6
PEN2	CAZ, CXM, GEN, KAN, ATM, CIP, TET	0.7
PEN3	CAZ, CXM, GEN, KAN, ATM, TET	0.6
PEN4	CAZ, CXM, GEN, KAN, ATM, TET	0.6
PWA1	CAZ, GEN, ATM, MEM	0.4
PWA2	CAZ, CXM, GEN, ATM, CIP, TET	0.6
PWA3	CAZ, CXM, GEN, KAN, TET	0.5
PIKP1	PTZ, CAZ, GEN, KAN, ATM	0.5
PIKP2	CAZ, CXM, GEN, KAN, ATM, TET	0.6
PIKP3	CAZ, GEN, ATM, MEM, TET	0.5
PEG1	CAZ, GEN, ATM, MEM, TET	0.5
PEG2	CAZ, CXM, ATM, MEM, TET	0.5
PEG3	CAZ, CXM, GEN, KAN, ATM, TET	0.6
PEG4	CAZ, CXM, GEN, KAN, ATM, TET	0.6

PTZ: Piperacillin; CAZ: Ceftazidime; ATM: Aztreonam; TET: Tetracycline; KAN: Kanamycin; ETP: Ertapenem; GEN: Gentamycin; MEM: Meropenem; CXM: Cefuroxime; CIP: Ciprofloxacin; PDR: *Pseudomonas* from drains; PEN: *Pseudomonas* from environment; PWA: *Pseudomonas* from wash water; PIKP: *Pseudomonas* from Ikpoba Okah; PEG: *Pseudomonas* from Egor.

Table 4
Multidrug resistance profile of the aquaculture and abattoir isolates.

Antimicrobial class	Number of antibiotics	Resistance phenotype	Number of isolates (*n* = 96)
4	5	PTZ, CAZ, GEN, KAN, ATM	8
4	5	PTZ, CAZ, GEN, KAN, ATM	12
4	4	CAZ, GEN, ATM, MEM	16
4	6	CAZ, CXM, GEN, KAN, ATM, TET	20
3	5	CAZ, CXM, GEN, KAN, ATM	28
2	4	CAZ, CXM, GEN, KAN	36
2	3	CAZ, CXM, GEN	40

PTZ: Piperacillin; CAZ: Ceftazidime; ATM: Aztreonam; TET: Tetracycline; KAN: Kanamycin; GEN: Gentamycin; MEM: Meropenem; CXM: Cefuroxime.

4. Discussion

The poor management practices adopted in abattoirs can affect the safety of meat in various ways, such as diseases in animals (zoonotic diseases), chemical (pesticides and antibiotics) residues deposition, most importantly by microbial contamination with pathogenic microbes and toxins[20]. Microbial populace are always in constant evolution, with unlimited adaptive capacities towards conditions that are used to inhibit/kill them, all making food safety a long-lasting issue. Ignorance concerning food safety measures provides potentials for increased food borne diseases in man[21]. Due to the diversity of *P. aeruginosa,* they are found in diverse ecological niches. Under stressful environmental conditions, these opportunistic microbial populations may become pathogenic, invade the body tissue and initiate infections. It is thus imperative to regulate and monitor the bacterial parameters in the current aquaculture and abattoir system where lots of profit maximization is done to enhance production.

Significant differences were observed in the mean pseudomonads count from Ikpoba-Okha, Egor and drains, environments, and wash water in May, June and July ($P < 0.05$). This could be attributed to the fact that both the abattoir and the pond samples are contaminated from the handling and preparation process of the butchers who dress and slaughter their products in contact with the waste deposited around them thereby making the final products unsafe for consumption and favoring the proliferation of *P. aeruginosa* as those

observed in the present study. Similar antibiotic resistance profile was also detected by Odjadjare *et al.*[5] where high level resistance was observed against the penicillins (90%–100%), cephems (70%), sulphamethoxazole (90%) and rifampin (90%). Also, Falodun and Adekanmbi[22] revealed 100% resistance to ceftazidime and piperacillin.

P. aeruginosa is being acknowledged as an increasing emerging organism causing opportunistic disease of clinical importance. A significant characteristic to public health it possesses is its reduced antibiotic susceptibility[8]. This reduced susceptibility is usually connected to a strenuous action of multiple antibiotic efflux pumps coupled with chromosomally mediated antibiotic resistance determinants (such as *mexXY*, *mexAB-oprM*) and the reduced permeability of the bacterial cellular envelopes by antimicrobial agents[3]. Aside intrinsic resistance, *P. aeruginosa* can easily develop acquired resistance resulting from the change in the genetic make-up in chromosomally encoded determinants or by the horizontal determinants transfer of antibiotic resistance genes[7]. Expansion of MDR by *P. aeruginosa* strains requires several different genotypic measures that include acquisition of horizontal dissemination of antibiotic resistance determinants and/or mutation[5]. Hyper-mutation enhances the choice of mutation-driven antibiotic resistance in *P. aeruginosa* strains consequently resulting in chronic infections, while the assembling of several diverse antibiotic resistance determinants in integrons enhances the acquisition of antibiotic resistance genes[7].

Antibacterial resistance is at present one of the significant threat to mankind as it constitutes alarming health crisis. Extensive uses of antibacterial agents in veterinary, human medicine and agricultural settings have led to the emergence of antibacterial-resistant organisms in different environmental settings including abattoir and aquaculture. Antibacterial resistance in aquaculture is a worldwide concern because antibacterial resistance genes can be disseminated easily from aquaculture and abattoir settings to other ecosystems and the food chain[13]. African catfish aquaculture has increased at a phenomenal rate through a continuous process of expansion, intensification, and diversification as a consequence of the Nigerian government sponsoring/financing small and medium scale enterprise. Risks of pathogenic bacterial isolates have also increased resulting in the increased use of antibacterial agents for therapy[23].

Antibacterial resistance in aquaculture and abattoir has significantly impacted the food chain and thus represents the risk to human and animal health[23]. Curbing antibacterial resistance, understanding of the sources, mechanisms and magnitude of antibacterial resistance and its significant impact on the food chain are important in designing, prioritizing control and monitoring programs that may generate data that would be relevant for performing implementation of antibacterial stewardship plans, quantitative microbial risk assessments, developing effective treatment regimen for the control of aquaculture and abattoir diseases and reducing risk to public health.

Similar investigation by Odjadjare *et al.*[5] on MAR index from effluent water ranged between 0.26 and 0.58. However, no clear pattern of MAR along the lines of isolate origin was observed. MDR has been described as acquired resistance to one agent in 3 or more antimicrobial classes, while extensive drug resistance has been described as resistance to one agent in all but 2 or fewer antimicrobial categories (*i.e.* bacterial isolates remain resistant to only 1 or 2 categories)[24].

It has been previously reported that antimicrobials are widely used in food animal production settings for treatment/prevention and for growth promotion[9,11]. Studies have also revealed that 50%–90% of drugs used to treat food producing animals are released into the environment either as metabolic or un-metabolized intermediates which, even though inactive, may go through transformation to the active form in the environment[7], consequently resulting in high selection of MDR *P. aeruginosa* from aquaculture and abattoir effluent.

The occurrence of MDR *P. aeruginosa* on food products could pose significant public health risks to consumers of such products. First, these include consumption of products contaminated with MDR bacteria which could result in infections as a consequence of antibiotic therapy which can be compromised due to resistant strain resulting in infections difficult to treat. However, it should be known that even if the products are cooked, the bacterial isolates might die off but it will not destroy determinants responsible for the resistance. Secondly, resistant pathogenic bacteria in animals can be transmitted to human via consumption of contaminated food products and resistance genes can also be disseminated to other bacteria via mobile genetic element such as integrons, plasmids, insertion sequence, transposons and gene cassette[2]. Finally, residual antibiotics in food animal products such as in the blood and liver can also result in the transmission of antibiotic resistant bacteria to the final consumer.

The potential pathogenic nature of *Pseudomonas* isolates is beyond doubt as different reports support findings in the present study. It was thus customary from the findings of our study that effluents and products from the abattoir and aquaculture environment could be an origin of dissemination of antibiotic resistant bacteria between human and animal population, and the environment. Therefore, public health workers must make efforts to regulate and monitor the bacterial parameters in the aquaculture and abattoir system to deter this trend to enhance qualitative production and prevent public health implication.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors wish to acknowledge The World Academy of Science, Italy for the financial support for this study (Grant No. 14-091 RG/BIO/AF/AC_1-UNESCO FR: 324028575). Dr. Etinosa Ogbomoede Igbinosa expresses gratitude to the Govan Mbeki Research and Development Centre of the University of Fort Hare, Alice, South Africa, for the facilities provided in course of preparing this manuscript.

References

[1] Akinyeye AJ, Aborisade WT, Akinmolayan AO. Bacteriological evaluation of abattoir effluent contaminated soil in Ikpoba, Benin, Nigeria. *Res Rev J Basic Sci* 2013; **1**(1): 1-6.

[2] Igbinosa IH, Igbinosa EO, Okoh AI. Molecular detection of metallo-β-lactamase and putative virulence genes in environmental isolates of *Pseudomonas* species. *Pol J Environ Stud* 2014; **23**(6): 2327-31.

[3] Igbinosa EO, Odjadjare EE, Igbinosa IH, Orhue PO, Omoigberale MN, Amhanre NI. Antibiotic synergy interaction against multidrug-resistant *Pseudomonas aeruginosa* isolated from an abattoir effluent environment. *ScientificWorldJournal* 2012; **2012**: 308034.

[4] Ogbomida ET, Kubeyinje B, Ezemonye LI. Evaluation of bacterial profile and biodegradation potential of abattoir wastewater. *Afr J Environ Sci Technol* 2016; **10**(2): 50-7.

[5] Odjadjare EE, Igbinosa EO, Mordi R, Igere B, Igeleke CL, Okoh AI. 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* 2012; **9**(6): 2092-107.

[6] Yah SC, Eghafona NO, Enabulelem IO. Prevalence of plasmids mediated *Pseudomonas aeruginosa* resistant genes from burn wound patients at the University of Benin Teaching Hospital Benin City, Nigeria. *J Med Biomed Sci* 2006; **5**(2): 61-8.

[7] Igbinosa EO, Obuekwe IS. Evaluation of antibiotic resistant gene in abattoir environment. *J Appl Sci Environ Manag* 2014; **18**(2): 165-70.

[8] Igbinosa EO, Odjadjare EE. Antibiotics and antibiotic resistance determinants: an undesired element in the environment. In: Méndez-Vilas A, editor. *The battle against microbial pathogens: basic science, technological advances and educational programs*. Vol. 2. Extremadura: Formatex Research Center; 2015, p. 858-66.

[9] Igbinosa EO, Beshiru A, Akporehe LU, Ogofure AG. Detection of methicillin-resistant *Staphylococci* isolated from food producing animals: a public health implication. *Vet Sci* 2016; **3**(3): 14.

[10] Isibor JO, Uwabor C. Antibiotic susceptibility pattern and plasmid profiles of *Pseudomonas aeruginosa* isolated from some hospital patients in Benin City, Nigeria. *New York Sci J* 2015; **8**(7): 14-9.

[11] Igbinosa EO, Beshiru A, Akporehe LU, Oviasogie FE, Igbinosa OO. Prevalence of methicillin-resistant *Staphylococcus aureus* and other *Staphylococcus* species in raw meat samples intended for human consumption in Benin City, Nigeria: implications for public health. *Int J Environ Res Public Health* 2016; **13**(10): 949.

[12] Wogu MD, Maduakor, CC. Evaluation of microbial spoilage of some aquacultured fresh fish in Benin City Nigeria. *Ethiop J Environ Stud Manag* 2010; **3**(3): 18-22.

[13] Chuah LO, Effarizah ME, Goni AM, Rusul G. Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. *Curr Environ Health Rep* 2016; **3**(2): 118-27.

[14] Ojiezeh TI, Ophori EA, Eghafona NO, Okiki PA. Prevalence of microflora in trachea of NDV challenged broiler following supplementation with extracts of *Aloe vera*, *Alma millsoni*, *Archachatina marginata* and *Ganoderma lucidum*. *J Nat Sci Res* 2013; **3**(9): 106-11.

[15] Atuanya EI, Nwogu NA, Akpor EA. Effluent qualities of government and private abattoirs and their effects on Ikpoba River, Benin City, Edo State, Nigeria. *Adv Biol Res* 2012; **6**(5): 196-201.

[16] Ikhajiagbe B, Kekere O, Omoregbee O, Omokha FI. Microbial and physiochemical quality of effluent water from a brewery in Benin City, Midwestern Nigeria. *J Sci Res Rep* 2014; **3**(3): 514-31.

[17] Atuanya EI, Edefetah MA, Nwogu NA. Microbiological qualities and some heavy metals (mercury and cadmium) levels of fresh and dry fish species sold in Benin City, Edo State, Nigeria. *Bull Environ Pharmacol Life Sci* 2011; **1**(1): 10-4.

[18] Lutz JK, Lee J. Prevalence and antimicrobial-resistance of *Pseudomonas aeruginosa* in swimming pools and hot tubs. *Int J Environ Res Public Health* 2011; **8**(2): 554-64.

[19] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fourth international supplements. Wayne: Clinical and Laboratory Standards Institute; 2014.[Online] Available from: http://www.gxccl.com/download/upload/CLSIM100-S24%E8%8B%B1%E6%96%87%E7%89%88.pdf [Accessed on 25th July, 2016]

[20] Abiade-Paul CU, Kene IC, Chah KF. Occurrence and antibiogram of *Salmonellae* in effluent from Nsukka Munincipal Abattoir. *Niger Vet J* 2005; **27**: 48-53.

[21] Nwanta JA, Onunkwo JI, Ezenduka VE, Phil-Eze PO, Egege SC. Abattoir operations and waste management in Nigeria: a review of challenges and prospects. *Sokoto J Vet Sci* 2008; **7**(2): 60-7.

[22] Falodun OI, Adekanmbi AO. Antibiogram of *Escherichia coli* and *Pseudomonas* strains isolated from wastewater generated by an abattoir as it journeys into a receiving river. *Adv Microbiol* 2016; **6**: 303-9.

[23] Anyanwu MU, Chah KF. Antibacterial resistance in African catfish aquaculture: a review. *Not Sci Biol* 2016; **8**(1): 1-20.

[24] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**(3): 268-81.