

Beta-lactamase Genes in Multi-resistant *Aeromonas* spp. isolated from River and Aquaculture Water Sources in Nigeria

*¹Chikwendu, C. L., ²Egbadon, E. O., ³Amadi, E. S., ⁴Ibe, S. N. and ⁵Okpokwasili, G. C.

¹Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, Nigeria

²Department of Microbiology, School of Biological Sciences, University of Port Harcourt, Port Harcourt, Nigeria

Abstract: This study examined multi-resistant *Aeromonas* spp. isolated from river and aquaculture water sources for determinants of resistant genes. These species can provide a reservoir for resistant genes capable of transfer to other water-borne and human pathogens. The isolates were confirmed with API 20NE. Resistance profiles of 206 *Aeromonas* isolates were determined for 11 antimicrobials by the Kirby-Bauer technique. PCR was used to determine the genetic determinants responsible for the ESBL phenotypes using primers for bla_{ppvi}, bla_{shv} and bla_{crx} beta-lactamase genes. Phenotypic expression of ESBL production was done by the double disk diffusion method and plasmid curing was effected with acridine orange. The *Aeromonas* spp. comprised of the following: *Aeromonas hydrophila/caviae*, *A. sobria* and *A. salmonicida*. Isolates expressed high resistant rates (75-100%) to 63.6% of the antimicrobials and moderate resistant rates (53.3-60.0%) to 27.3% of the antimicrobials tested. There were also high occurrences of multiple resistances with 100% of the isolates being resistant to 2 or more antimicrobials. *Aeromonas* spp. from river water expressed higher resistant rates than those from aquaculture water samples. Phenotypic screening for carriage or presence of ESBL gene showed that all the isolates tested positive for the presence of ESBL gene and were resistant to amoxicillin/clavulanate. Amongst the 13 isolates analysed for the 3 beta-lactamase genes, the bla_{TEM} was most prevalent with 30.8% of isolates possessing it, while 23.1% and 7.7% possessed bla_{shv} and bla_{crx} respectively. Antimicrobial resistance profile, post curing, showed 38% and 100% of isolates remained ESBL producers and inhibitor resistant respectively. The study infers the presence and diversity of ESBL genes in *Aeromonas* spp. isolated from river and aquaculture water settings in Nigeria.

Keywords: antimicrobial resistance, beta-lactamase, plasmid

Introduction

The genus *Aeromonas* consists of bacteria that are Gram-negative rods and are considered ubiquitous in aquatic environments (Janda and Abbott, 2010), since they have been found to inhabit surface water (river, lakes), sewage, drinking water (tap, bottled and mineral) (Abulhamd, 2010; Pablos *et al.*, 2011; Odeyemi *et al.*, 2012), thermal waters and sea waters (Biscardi *et al.*, 2002; Maalej *et al.*, 2003; Pablos *et al.*, 2009). Some species, mainly the psychrophilic *Aeromonas salmonicida* and the mesophilic *Aeromonas hydrophila* and *Aeromonas veronii* are known causative agents of fish disease (Janda and Abbott, 2010) and are also important human opportunistic pathogens with ability to cause various types of diseases, including intestinal, blood, skin and soft tissue and trauma-related infections (Vivekanandhan *et al.*, 2002; Aminov, 2009; Lamy *et al.*, 2009; Janda and Abbott, 2010).

Aeromonas spp. have also been found to be environmental reservoirs of resistance determinants to different classes of antibiotics (Cattoir *et al.*, 2008; Goni-Uribe *et al.*, 2000a; L'Abée-Lund and Sørum 2000). According to Hernould *et al.* (2008), intrinsic resistance to beta-lactams among the genus may arise from the expression of chromosomal beta-lactamases and/or efflux pumps. In addition to that, environmental

contamination with antibiotics and other pollutants also contribute to the maintenance and spread of antibiotic resistance genes (Goni-Uribe *et al.*, 2000a). According to Kruse and Sørum (1994), one mechanism that allows the perpetuation of such genes is the spread of resistance plasmids between unrelated bacteria in natural environments. Bacteria with intrinsic or acquired resistance to antibiotics are commonly found in aquatic environments, where *Pseudomonas*, *Serratia* and *Aeromonas* are commonly identified.

Aeromonas spp., non-cholera vibrios and *Plesiomonas shigelloides* belong to the expanding group of water and food borne pathogens. They are widely distributed in aquatic environments and are increasingly regarded as important pathogens of aquatic animals, causing significant economic losses in the aquaculture industry worldwide. In addition, these bacteria have been implicated as opportunistic pathogens mainly causing gastroenteritis in humans (Austin and Austin, 1993).

This study was therefore aimed at identifying the antimicrobial resistance profile of multiple resistant *Aeromonas* isolates from river and aquaculture water sources as well as the presence of beta-lactamase enzyme determinants among the multiple resistant isolates.

*Corresponding author:

chinwe.chikwendu@futo.edu.ng ¹Chikwendu, C. L.,
Copyright © 2017 Nigerian Society for Microbiology

Materials and Methods

Sample types and processing

River and aquaculture water samples for analyses were collected with sterile 1L sample

containers. Initial bacterial isolation was carried out by placing an aliquot (0.1ml) each of river and aquaculture water samples on the dry surface of the *Pseudomonas* – *Aeromonas* (GSP agar, Merck) selective agar plates and spread round the plates using sterile hockey sticks. Plates were then turned upside down, labelled and incubated at $28 \pm 2^\circ\text{C}$ for 24 – 48 hours. *Aeromonas* isolates were bright yellow in colour on the *Pseudomonas* – *Aeromonas* selective agar plates. Two or three well-spaced colonies per plate were then picked, purified and confirmed with API 20 NE (Bio-Mérieux).

Detection of antimicrobial resistant phenotypes

Antimicrobial phenotypes were determined by the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar (Oxoid, Basingstoke). The antibiotics assayed were: ampicillin (10 µg), mezlocillin (75 µg), streptomycin (10 µg), cefuroxime (30 µg), ofloxacin (5 µg), cefotaxime (30 µg), cotrimoxazole (25 µg), gentamicin (120 µg), ceftazidime (30 µg), enrofloxacin (5 µg) and ciprofloxacin (5 µg) (Oxoid, Basingstoke). Resistance results were interpreted according to CLSI standards (CLSI 2007). The double disk diffusion technique was used to determine phenotypic expression of ESBL production by the isolates.

Plasmid isolation, profiling and curing

Plasmid extraction was performed using the method of Ehrenfeld and Clewell (1987) and profiling was carried out on 0.8% Agarose gel in a 0.5% concentration of Tris-Borate-EDTA (TBE) buffer. A HIND III digest of λ DNA was used as molecular

weight marker and the gel was electrophoresed in a horizontal tank at a voltage of 60V for 1h 30min. Plasmid DNA bands were identified by fluorescence of bound ethidium bromide using a short wave ultra violet light transilluminator. Photographs were taken with a digital camera. Thirteen (13) representative isolates were chosen and analysed for the presence or otherwise of plasmids.

Plasmid curing was carried out by the method of Salisbury et al. (1972), using acridine orange dye.

PCR detection of β -lactamase genes

Aeromonas genomic DNA was extracted by the alkaline lyses method of Birboim and Doly (1979). Bacterial chromosomal DNA was used as substrate for the PCR and specific primers for the β -lactamase genes TEM, SHV and CTX were used for the detection of resistance genes (Table 1). PCR was performed in 25 µl of a reaction mixture containing DNA (10-200ng), 200 µM of each deoxynucleoside triphosphates (dNTP) (Promega), 1.5 mM MgCl_2 , 1X PCR Buffer, 20 pMol (each) of the primers, 1 unit of *Taq* DNA polymerase (Promega) and sterile distilled water. Thermal cycling was conducted in an Eppendorf Master Cycler Gradient at an initial denaturation temperature of 94°C for 5 minutes, followed by 30 amplification cycles of 1 minute at 94°C ; 1 minute at (50°C for blaTEM, 54°C for blaSHV and 62°C for blaCTX), and 1 minute at 72°C . This was followed by a final extension step of 10 minutes at 72°C . The amplification product was separated on 1.5% agarose gel and visualized by staining with ethidium bromide. One hundred (100) bp DNA ladder was used as DNA molecular weight standard.

Table 1: The primers used to screen for ESBL genes

Primer	Gene	Sequence/size (bp)	Reference
757(FP)	blaTEM	5'-GCGGAACCCCTATTTG-3' / 964	Olesen et al., (2004)
821(RP)	blaTEM	5'-TCTAAAGTATATATGAGTAACTTGGTCTGAC-3' / 964	Olesen et al., (2004)
1436(FP)	blaSHV	5'-TTTCGCTGTGTATTATCTCCCTG-3' / 854	Hasman et al., (2005)
1437(RP)	blaSHV	5'-TTAGCGTTGCCAGTGYTCG-3' / 854	Hasman et al., (2005)
1354(FP)	blaCTX	5'-ATGTGCAGYACCAGTAARGTKATGGC-3' / 593	Miro et al., (2002)
1355(RP)	blaCTX	5'-TGGGTRAARTARGTSACCAGAAACAGCGG-3' / 593	Miro et al., (2002)

KEY: FP - Forward primer; RP - Reverse Primer

Results and Discussion

A total of 206 isolates of *Aeromonas* spp. were recovered from both 100 river and aquaculture water samples. The *Aeromonas* spp. comprised of the following: *Aeromonas hydrophila/caviae*, *A. sobria* and *A. salmonicida*, as confirmed by API 20NE.

Results of screening against eleven (11) antimicrobials showed that the isolates expressed high resistances to Ampicillin, mezlocillin, streptomycin, cefuroxime, ofloxacin, cefotaxime and cotrimoxazole

(75.0-100.0%), resistances to gentamycin, ceftazidime and enrofloxacin were moderate, (53.3-60.0%), while resistance to ciprofloxacin was low (39.9%) as shown in Table 1. There were significant differences ($P=0.05$) in resistance between isolates from the river and aquaculture water sources.

The *Aeromonas* spp. isolated expressed high occurrences of multiple resistances, with 100% of the organisms being resistant to at least 2 or more antimicrobials for both sample types (Figure 1).

Aeromonas spp. isolated from river water expressed greater resistance than those isolated from aquaculture water for the same antimicrobials. However, both river water and aquaculture water isolates expressed the least resistance rates to ciprofloxacin (Figure 2).

Two plasmids of sizes 639bp and 1110bp were isolated from one *Aeromonas* isolate, out of 13 isolates analysed for the presence of plasmids. Two aquaculture and 4 river water isolates showed amplification for the 3 β -lactamase genes tested, with *bla*_{TEM} being the most prevalent at 30.8%, while 23.1% and 7.7% of the isolates possessed *bla*_{SHV} and *bla*_{CTX} β -lactamase genes respectively (Table 2; Figures 2 and 3).

Fifty-one resistance patterns were identified amongst the *Aeromonas* spp. isolated from the river water samples, while 49 resistance patterns were identified amongst the isolates from aquaculture water samples (Table 3).

Aeromonas spp. are known to be intrinsically susceptible to all antibiotics active against non-fastidious gram negative bacilli except for many β -lactams, due to the production of multiple inducible chromosomally encoded β -lactamases (Jones and Wilcox, 1995; Rossolini et al., 1996). In the present study, all *Aeromonas* spp. isolated were resistant to Ampicillin, this is similar to the observation of Goni-Urizza et al. (2000a) and Odeyemi and Ahmad (2017). Ampicillin is inactivated by chromosomal beta-lactamases produced by many enterobacteriaceae and *Aeromonas* (Walsh et al., 1995). This supports the assertion that *Aeromonas* is naturally resistant to ampicillin (Walsh et al., 1995). They were also highly resistant to cefuroxime (85.9%) and cefotaxime (76.7%).

Resistance to ceftazidime was moderate at 57.8%. Resistance to third generation cephalosporins is known to be associated with the de-repression of the chromosomal enzyme (Jones and Wilcox, 1995; Rossolini et al., 1996). According to Jones and Wilcox (1995), β -lactam agents should be avoided in the treatment of *Aeromonas* spp. infections even if MICs are still in the susceptible range, since resistant mutants over producing their chromosomal β -lactamases may be selected during therapy.

The *Aeromonas* spp. isolated were also poorly susceptible to streptomycin, similar to the results of Goni-Urizza et al. (2000a). About 75% of the isolates were also resistant to co-trimoxazole in contrast to the assertion that the agent is generally efficient at eliminating *Aeromonas* due to the strong synergy between the drugs (Jones and Wilcox, 1995). The trend however is similar to that of Ko et al. (1996), who reported 39 – 50% resistance to co-trimoxazole for his isolates.

The high rate of resistance to cefuroxime and ofloxacin among the environmental *Aeromonas* isolates in this study is in contrast to Huddleston et al. (2006), whose isolates showed no resistance to cefuroxime and ofloxacin. Some other results, (Kämpfer et al., 1999;

Overman and Janda, 1999; Vila et al., 2002; Warren et al., 2004) also showed no or low incidence of resistance with cefuroxime and ofloxacin. However, Goni-Urizza et al. (2000b) found resistance to ofloxacin in 59% of his isolates. Generally, the environmental *Aeromonas* isolates from the river and aquaculture water samples in this study expressed very high levels of resistance to the test antimicrobials. A possible explanation for these findings could be that some of the isolates may include clinical isolates that could have been previously exposed to antibiotics (Kämpfer et al., 1999; Ko et al., 1996; Vila et al., 2002).

In other studies carried out, aeromonads were isolated from waters that were highly polluted by industrial effluent or raw sewage (Goni-Urizza et al., 2000b). The samples in this study were collected from river water sources that received both human and animal sewage. Domestic activities like bathing and washing of clothes were some of the activities carried out near the river water sources. One of the sampling points on the river also receives effluent discharge from the Federal Medical Centre, Owerri. Aquaculture fishes are also routinely fed with waste materials including chicken droppings and sewage.

The *Aeromonas* isolates expressed high incidences of multiple resistances with 100% of the isolates being resistant to at least 2 or more antimicrobials for both the river water and aquaculture samples. Previous studies have also shown the occurrence multiple resistances in *Aeromonas* (Miranda and Castillo, 1998; Odeyemi and Ahmad, 2017). The isolates exhibited a large number of resistance patterns (51 and 49 amongst the river water and aquaculture isolates respectively), which is an indication of the high variability in character associated with these environmental isolates.

Plasmids have been detected in *Aeromonas* spp. as reported previously in other studies (Akinbowale et al., 2006; Rhodes et al., 2000; Furushita et al., 2003). Similar results include plasmids in 33.3% of *Aeromonas* sp from tilapia skin lesions (Son et al., 1997), plasmid-associated ampicillin and tetracycline resistance associated with a plasmid in a single strain only (Son et al., 1997), and 56.6% overall prevalence of plasmids in fresh water fish isolates (Radu et al., 2003). Some isolates in Jacobs and Chenia (2007) also appeared to carry multiple plasmids simultaneously as in the present study. According to Jacobs and Chenia (2007), this multiplicity and intensity of plasmids harboured by the *Aeromonas* isolates may be related to their copy number in the host cell.

Aeromonas spp. are known to harbour self – transmissible (Sørum et al., 2003) and non – conjugative but mobilizable plasmids (L'Abée-Lund and Sørum, 2002) carrying antimicrobial resistance determinants and their associated mobile genetic elements. Thus the exchange of genetic information between *Aeromonas* spp. isolates is not unlikely, and

might occur with neighbouring microflora in environmental and clinical niches (Rhodes *et al.*, 2000).

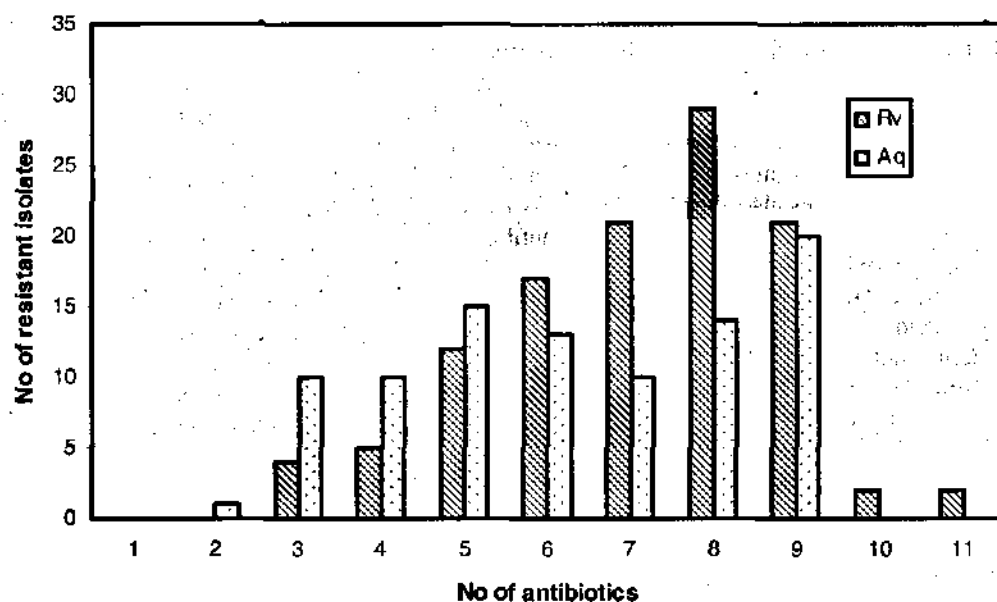
Six of the 13 isolates analysed for the *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX} beta-lactamase genes were found to possess them in different proportions, with *bla*_{TEM} being the most predominant. A post curing analysis of antimicrobial resistance of the isolates also showed that all the *Aeromonas* isolates were inhibitor resistant, being resistant to both amoxicillin-clavulanic acid, while 15.4% of the isolates remained resistance to cefotaxime-clavulanic acid. In previous studies, resistance to β -lactam and β -lactamase inhibitor combinations in *Escherichia coli* has been reported to be due to hyper-production of class A β -lactamases, like TEM-1 or SHV-1, class D plasmid-mediated enzyme or chromosomal or plasmidic class C β -lactamase and/or to modified outer membrane permeability (Reguera *et al.*, 1991; Oliver *et al.*, 1999; Chaibi *et al.*, 1999; Thomas and Moland, 2000).

According to Schmidt *et al.* (2001), the presence of a diversity of resistance genes amongst aeromonads might constitute a pool of resistance genes capable of moving among bacteria in the aquatic environment and possibly being transferred to other fish pathogens. Indeed the presence of resistance genes and associated mobile genetic elements in the absence of antibiotic pressure is a cause for concern (Jacobs and Chenia, 2007). The mobilization of these genes in the aquatic environment and transmission to the human compartment is not unlikely and might provide a reservoir of resistance genes capable of transfer to other water-borne and human pathogens (Schmidt *et al.*, 2001). Furthermore, the aquatic environment has been referred to as an important reservoir of novel antibiotic resistance genes (Cattoir *et al.*, 2008).

Table 1: Frequency of antimicrobial resistance of *Aeromonas* spp. isolated from river water and aquaculture sources.

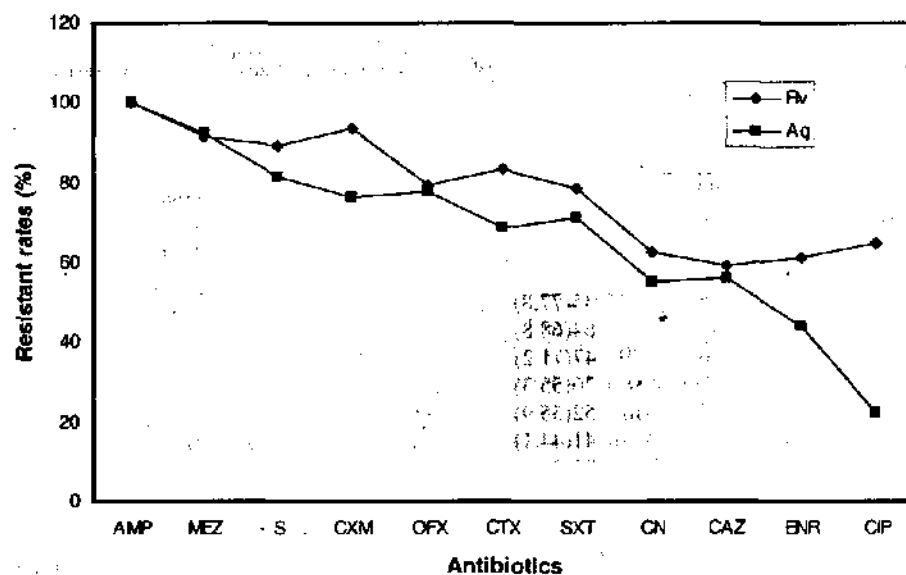
Source of environmental <i>Aeromonas</i> spp. isolates							
Antibiotics	River water samples		Aquaculture water samples		River and aquaculture samples		Total (%) resistance
	No. of isolates	No. (%) of resistant isolates	No. of isolates	No. (%) of resistant isolates	Total no. of isolates		
AMP	113	113(100)	93	93(100)	206		206(100)
MEZ	70	64(91.4)	66	61(92.4)	136		125(91.9)
S	113	101(89.4)	93	76(81.7)	206		177(85.9)
CXM	113	106(93.8)	93	71(76.3)	206		177(85.9)
OFX	58	46(79.3)	45	35(77.8)	103		81(78.6)
CTX	113	94(83.2)	93	64(68.8)	206		158(76.7)
SXT	70	55(78.6)	66	47(71.2)	136		102(75.0)
CN	83	52(62.7)	47	26(55.3)	130		78(60.0)
CAZ	113	67(59.3)	93	52(55.9)	206		119(57.8)
ENR	104	64(61.5)	93	41(44.1)	197		105(53.3)
CIP	65	42(64.6)	93	21(22.6)	158		63(39.9)

KEY: AMP, Ampicillin; MEZ, mezlocillin; S, streptomycin; CXM, cefuroxime; OFX, ofloxacin; CTX, cefotaxime; SXT, cotrimoxazole; CN, gentamycin; CAZ, ceftazidime; ENR, enrofloxacin; CIP, ciprofloxacin.

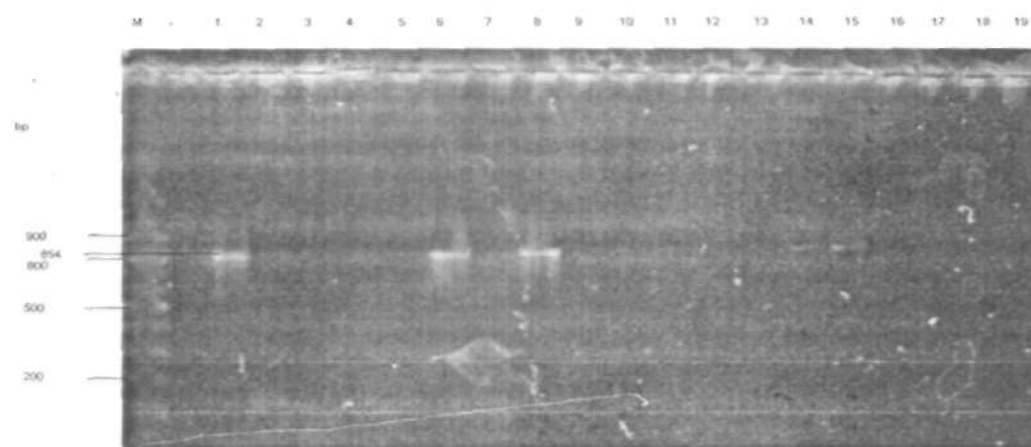


KEY: Rv, River water; Aq, Aquaculture water.

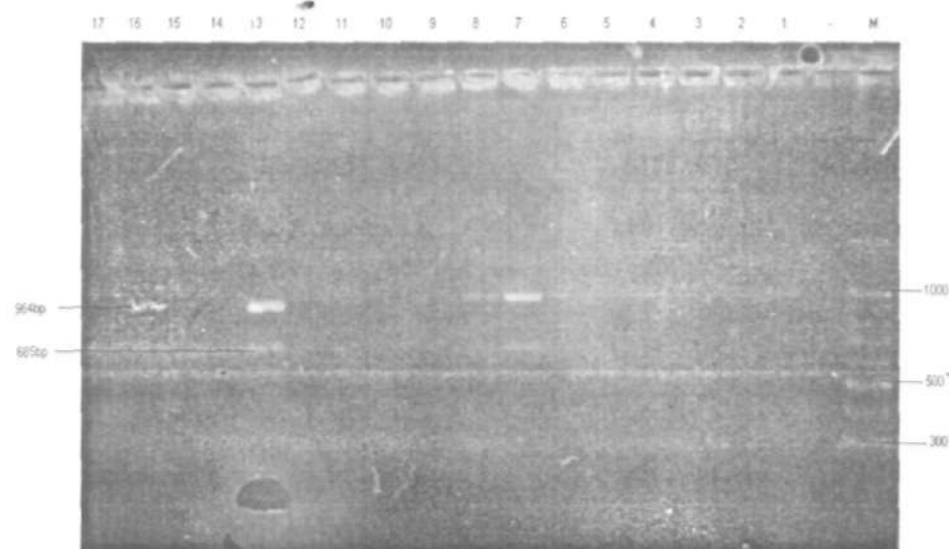
Figure 1: Multiple resistances in *Aeromonas* spp. isolated from river and aquaculture water samples



KEY: Rv, River water; Aq, Aquaculture water; AMP, Ampicillin; MEZ, mezlocillin; S, streptomycin; CXM, cefuroxime; OFX, ofloxacin; CTX, cefotaxime; SXT, cotrimoxazole; CN, gentamycin; CAZ, ceftazidime; ENR, enrofloxacin; CIP, ciprofloxacin.

Fig 2: Comparative rates of resistance in *Aeromonas* spp. isolated from river and aquaculture water samples

KEY: Lanes 1-13, PCR amplified products of *Aeromonas* spp. from river and aquaculture water; M, molecular weight marker.

Fig 3: Gel electrophoresis of the PCR amplified products for the detection of *bla*_{SHV} β -lactamase genes for isolates 1 – 13.

KEY: Lanes 1-13, PCR amplified products of *Aeromonas* sp from river and aquaculture water; M, molecular weight marker

Fig 4: Gel electrophoresis of the PCR amplified products for the detection of *bla*_{TEM} β -lactamase genes for isolates 1 – 17.Table 2: Amplification bands of *bla*_{SHV}, *bla*_{TEM} and *bla*_{CTX} β -lactamase positive isolates

Isolate no	Source	Name	β -lactamase gene amplification band (bp)		
			<i>bla</i> _{SHV}	<i>bla</i> _{TEM}	<i>bla</i> _{CTX}
1	Aquaculture	<i>Aeromonas</i> spp.	854	964	-
3	Aquaculture	<i>Aeromonas</i> spp.	-	-	593
6,10,11	River	<i>Aeromonas</i> spp.	854	-	-
7	River	<i>Aeromonas</i> spp.	-	685, 964	-
8	River	<i>Aeromonas</i> spp.	854	964	-
13	River	<i>Aeromonas</i> spp.	-	685, 964	-

Table 3: Antimicrobial resistance patterns of isolates

Isolate	Source	No. of patterns	Most predominant pattern	No. (%) of isolates with predominant pattern
<i>Aeromonas</i> sp	Rv	51	CAZ+CTX+CXM+ENR+CN+S+MEZ+OFX+AMP	19 (16.80)
<i>Aeromonas</i> sp	Aq	49	CAZ+CTX+CXM+ENR+SXT+MEZ+AMP+OFX	8 (8.60)
			CAZ+CTX+CXM+ENR+CN+S+CIP+AMP+OFX	8 (8.60)

KEY: Rv, river water; Aq, aquaculture; CAZ, ceftazidime; CTX, cefotaxime; CXM, cefuroxime; ENR, enrofloxacin; CN, gentamycin; S, streptomycin; MEZ, mezlocillin; OFX, ofloxacin; AMP, ampicillin; CIP, ciprofloxacin.

References

- Abulhamd, A. (2010). Genetic diversity and antimicrobial susceptibility of motile aquatic aeromonads. *Intl. J. Chem. Eng. Appl.* 1:90-95
- Akinbowale, O. H., Peng H. and Barton, M. D. (2006). Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *J. Appl. Microbiol.* 100: 1103-1113
- Aminov, R. I. (2009). The role of antibiotics and antibiotic resistance in nature. *Environ. Microbiol.* 11(12): 2970-2988
- Austin, B. and Austin, D. A. (1993). Bacterial fish pathogens In: *Diseases in Farmed and Wild Fish*. Chichester, U.K. Ellis Horwood.
- Birboim, H. C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7: 1513-1523.
- Biscardi, D., Casteldo, A., Gualillo, O. and Fuscus, R. (2002). The occurrence of cytotoxic *Aeromonas hydrophila* strains in Italian mineral and thermal waters. *Science Total Environ.* 292: 255-263
- Cattoir, V., Poirel, L., Aubert, C., Soussy, C. J. and Nordmann, P. (2008). Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental *Aeromonas* sp. *Emerg. Infect. Dis.* 14:231-237.
- Chaibi, E. B., Sirot, D., Paul, G. and Labia, R. (1999). Inhibitor-resistant TEM β -lactamases: phenotypic, genetic and biochemical characteristics. *J. Antimicrob. Chemother.* 43:447-458
- Ehrenfeld, E. E. and Clewell, D. B. (1987). Transfer functions of the *Streptococcus faecalis* plasmid pAD1: organization of plasmid DNA encoding response to sex pheromone. *J. Bacteriol.* 169: 3473-3481.
- Furushita, M. T., Shiōa, T., Maeda, T. Yahata, M., Kaneoka, A., Takahashi, Y., Torii, K. and Hasegawa, T. (2003). Similarity of tetracycline resistance genes isolated from fish farm bacteria to those from clinical isolates. *Appl. Environ. Microbiol.* 69:5336-5342.
- Goni-Uribe, M., Pineau, L., Capdepuy, M., Roques, C., Caumette, P. and Quentin, M. (2000a). Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. *J. Antimicrob. Chemother.* 46: 297-301.
- Goni-Uribe, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, P. and Quentin, C. (2000b). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* sp. *Appl. Environ. Microbiol.* 66:125-132
- Hasman H., Mevius, D., Veldman, K., Olesen, I. and Aarestrup, F. M. (2005). Beta-Lactamases among extended-spectrum β -lactamase (ES β -L)-resistant *Salmonella* from poultry, poultry products and human patients in the Netherlands. *J. Antimicrob. Chemother.* 56: 115-121.
- Hernould, M., Gagné, S., Fournier, M., Quentin, C. and Arpin, C. (2008). Role of AleABC efflux pump in *Aeromonas hydrophila* intrinsic multidrug resistance. *Antimicrob. Agents Chemother.* 52:1559-1563
- Huddleston, J. R., Zak, J. C. and Jeter, R. M. (2006). Antimicrobial susceptibilities of *Aeromonas* sp isolated from environmental sources. *Appl. Environ. Microbiol.* 72: 7036-7042.

- Jacobs, L. and Chema, H. Y. (2007). Characterization of integrons and tetracycline resistant determinants in *Aeromonas* sp isolated from South African aquaculture systems. *Intl. J. Food Microbiol.* **114**: 295-306.
- Janda, J. M. and Abbott, S. I. (2010). The genus *Aeromonas*: taxonomy, pathogenicity and infection. *Clin. Microbiol. Rev.* **23**:35-73
- Jones, B. L. and Wilcox, M. H. (1995). *Aeromonas* infections and their treatment. *J. Antimicrob. Chemother.* **35**: 453-461.
- Kämpfer, P., Christmann, C., Swings, J. and Huys, G. (1999). In vitro susceptibilities of *Aeromonas* genomic species to 69 antimicrobial agents. *Syst. Appl. Microbiol.* **22**: 662-669.
- Ko, W. C., Yu, K. W., Liu, C. Y., Huang, C. T., Leu H. S. and Chuang, Y. C. (1996). Increasing antibiotic resistance in clinical isolates of *Aeromonas* strains in Taiwan. *Antimicrob. Agents Chemother.* **40**: 1260-1262.
- Kruse, H. and Sørum, H. (1994). Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Appl. Environ. Microbiol.* **60**: 4015-4021.
- L'Abée-Lund, T. M. and Sørum, H. (2000). Functional Tn5393-like transposon in the plasmid pRAS2 from the fish pathogen *Aeromonas salmonicida* subspecies *salmonicida* isolated in Norway. *Appl. Environ. Microbiol.* **66**:5533-5535.
- L'Abée-Lund, T. M. and Sørum, H. (2002). A global non-conjugative TetC plasmid, pRAS3 from *Aeromonas salmonicida*. *Plasmid.* **47**: 172-181.
- Lamy, B., Kodjo, A. and Laurent, F., colBVH Study group. (2009). Prospective nationwide study of *Aeromonas* infections in France. *J. Clin. Microbiol.* **47**:1234-1237
- Maalej, S., Mahjoubi, A., Elazri, C. and Dukau, S. (2003). Simultaneous effects of environmental factors on motile *Aeromonas* dynamics in an urban effluent and in the natural sea water. *Water Research.* **37**:2865-2867
- Miranda, C. D. and Castillo, G. (1998). Resistance to antibiotic and heavy metals of motile aeromonads from Chilean fresh water. *Sci. Total Environ.* **224**: 167-176
- Miro, E., Navarro, F., Mirelis, B., Sabaté, M., Rivera, A., Coll, P. and Prats, G. (2002). Prevalence of clinical isolates of *Escherichia coli* producing inhibitor-resistant β -lactamases at a University Hospital in Barcelona, Spain, over a 3-year period. *Antimicrob. Agents Chemother.* **46**: 3991-3994.
- Odeyemi, O. A., Asmat, A. and Usup, G. (2012). Antibiotic resistance and putative virulence factors of *Aeromonas hydrophila* isolated from estuary. *J. Microbiol. Biotechnol. Food Sci.* **1**: 1339-1357
- Odeyemi, O. A. and Ahmad, A. (2017). Antibiotic resistance profiling and phenotyping of *Aeromonas* sp. isolated from aquatic sources. *Saudi J. Biol. Sci.* **24**(1):65-70
- Olesen I, Hasman, H. and Aarestrup, F. M. (2004). Prevalence of β -lactamases among ampicillin resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. *Microb. Drug Resist.* **10**: 334-340.
- Oliver, A., Perez-Vazquez, M., Martinez-Ferrer, M., Baquero, F., de Rafael, L. and Canton, R. (1999). Ampicillin-sulbactam and amoxicillin-clavulanate susceptibility testing of *Escherichia coli* isolates with different β -lactam resistance phenotypes. *Antimicrob. Agents Chemother.* **43**:707-714
- Overman, T. I. and Janda, J. M. (1999). Antimicrobial susceptibility patterns of *Aeromonas jandaei*, *Aeromonas schubertii*, *Aeromonas trota* and *Aeromonas veronii* biotype *veronii*. *J. Clin. Microbiol.* **37**: 706-708.
- Pablos, M., Rodriguez-Calleja, J. M., Santos, J. A., Otero, A. and Garcia-lopez, M. L. (2009). Occurrence of motile *Aeromonas* in municipal drinking water and distribution of genes encoding virulence factors. *Intl. J. Food Microbiol.* **135**:158-164
- Pablos, M., Huys, G., Crockeert, M., Rodriguez-Calleja, J. Otero, A., Santos, J. and García-López, M. (2011). Identification and epidemiological relationships of *Aeromonas* isolates from patients with diarrhea, drinking water and foods. *Intl. J. food Microbiol.* **147**:203-210.
- Radu, S., Ahmad, N., Ling, F. H. and Relzal, A. (2003). Prevalence and resistance to antibiotics for *Aeromonas* sp. from retail fish in Malaysia. *Intl. J. Food Microbiol.* **81**: 261-266.
- Reguera, J. A., Baquero, F., Perez-Diaz, J. C. and Martinez, J. L. (1991). Factors determining resistance to β -lactam combined with β -lactamase inhibitors in *Escherichia coli*. *J. Antimicrob. Chemother.* **27**: 569-575
- Rhodes, G., Huy, G., Swings, J., McGann, P., Hiney, M., Smith, P. and Pickup, R. W. (2000). Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline determinant Tet A. *Appl. Environ. Microbiol.* **66**: 3883-3890.
- Rossolini, G. M., T. Walsh, and G. Amicosante. (1996). The *Aeromonas* metallo-beta-lactamases: genetics, enzymology and contribution to drug

- resistance. *Microb. Drug Resistance*. 2: 245-252.
- Salisbury, V., Hedges, R. W. and Datta, N. (1972). Two modes of 'curing' transmissible bacterial plasmids. *J. Gen. Microbiol.* 70: 443-452.
- Schmidt, A. S., Bruun, M. S., Dalsgaard, I. and Larsen, J. L. (2001). Characterization of class 1 integrons associated with R-plasmids in clinical *Aeromonas salmonicidae* isolates from various geographical areas. *J. Antimicrobiol. Chemother.* 47: 735-743.
- Son, R., Rusul, G., Sahilah, A. M., Zainuri, A., Raha, A. R. and Salmah, I. (1997). Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, *Telapia* (*Telapia mossambica*). *Lett. Appl. Microbiol.* 24: 479-482.
- Sørum, H., L'Abée-Jund, T. M., Solberg, A. and Wold, A. (2003). Integron containing IncU R-plasmids pRASI and pAr-32 from the fish pathogen *Aeromonas salmonicida*. *Antimicrob. Agents Chemother.* 47: 1285-1290.
- Thomas, K. S. and Moland, E. S. (2000). Version 2000: the new β -lactamases of gram-negative bacteria at the dawn of the new millennium. *Microbes Infect.* 2: 1225-1235.
- Vila, J., Marco, F., Soler, L., Chacon, M. and Figueras, M. J. (2002). In vitro antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas veronii* biotype *sobria*. *J. Antimicrob. Chemother.* 49: 701-702.
- Vivekanandhan, G., Savithamani, K., Hatha, A. A. M., Lakshmanaperumalsamy, P. (2002). Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. *Int. J. Food Microbiol.* 76: 165-168.
- Walsh, T. R., Payne, D. J., MacGowan, A. P. and Bennett, P. M. (1995). A clinical isolate of *Aeromonas sobria* with three chromosomally mediated inducible beta-lactamases: a cephalosporinase, a penicillinase and a third enzyme displaying carbapenemase activity. *J. Antimicrob. Chemother.* 35: 271-279.
- Warren, W. J., Jeter, R. M., Kimbrough, R. C. and Zak, J. C. (2004). Population patterns and antimicrobial resistance of *Aeromonas* in urban playa lakes. *Can. J. Microbiol.* 50: 397-404.