Occurrence and Antibiotic Susceptibility of *Aeromonas* species from Piggery Farms in Ebonyi State, Nigeria

Onuoha, S .C*[1,2], Eronmosele, B. O.,[2] Okoh, F.N.,[2] Okafor, C. O.,[2] Onwere, C.C.[2] and Ovia, K. N.[2]

[1]Department of Biotechnology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

[2]Department of Microbiology, College of Sciences, Evangel University Akaeze, Ebonyi State, Nigeria.

[3]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

*Correspondence: sconuoha@yahoo.com; Tel: +2348032385682; Orcid Number: (https://orcid.org/ 0000-0002-6076-3910

Abstract: *Aeromonas* has been implicated as the most common bacterial disease in farm animals. In Ebonyi State, Nigeria, these infections and their adverse effects on public health have not been well investigated. The study detects the presence of *Aeromonas* species from Piggery Farms and determines the antibiotic resistance profile of the isolates. A total of 50 fresh Pig feacal samples were randomly collected from 25 different piggery farms in various parts of State. Bacterial detection was conducted using cultural methods and biochemical analysis. Susceptibility of the *Aeromonas* spp to antimicrobials was investigated using the Kirby−Bauer disk diffusion method. The study revealed that the microbial load ranges from $5.8\pm2.2x10^6$ CFU/mL to $22.0\pm9.6x10^6$ CFU/mL across the locations. The isolates showed 100% resistance to amoxicillin and cefuroxime followed by cefotaxine (90 %), tobramycin (80 %), ceftazidine (70 %), meropenem (60 %), The isolates are more susceptible to imipenem (90 %), followed by cefepime (60 %). All the isolates were resistant to at least three classes of antimicrobials, and had a multiple antibiotic resistance index score between 0.5 and 0.9. The study demonstrated that pig farms are potential public health threat as they are found to be contaminated with high bacterial load of *Aeromonas* spp which exhibited resistance to some of the life-saving antibiotics. The study advocates good management practices and successful control approaches to protect consumers and to minimize the risk of drug resistance.

Keywords: Antibiotics, Susceptibility, *Aeromonas* spp, Piggery Farms

INTRODUCTION

Aeromonas is a Gram-negative, facultative anaerobic, rod-shaped bacteria that morphologically resemble members of the family Enterobacteriace. The genus is made up of psychrophiles and mesophiles from soil and aquatic environments and causes different kinds of diseases to many warm and cold-blooded animals (Dascklov, 2013). Until late 1970s, Aeromonads were classified into two main groups based on physiological properties and host range until the late 1970s. The optimum growth temperature for motile aeromonads is between 35 and 37°C whereas non-motile aeromonads grows best at 22-23°C. Phenotypic markers for their differentiation include optimum growth temperature, motility, production of indole, and elaboration of a melanin-like pigment on tyrosine agar (Joseph and Carnahan, 2015). The genus Aeromonas has advanced with the addition of new species and the reclassification of preexisting taxa (Neyts *et al.*, 2014).

Aeromonas spp. have been implicated in a range of diseases in animals and humans, including gastroenteritis, septic arthritis, peritonitis, osteomyelitis, myositis, ocular infections, meningitis, cholangitis, pneumonia, hemolytic uremic syndrome, and urinary tract infections (Liao *et al*, 2010, Cabié *et al.*, 2010, Gowda *et al*., 2015).

Members of this genus are cosmopolitan across numerous ecosystems. *A. veronii* has been isolated from the environment (air, water and soil), food animals (shellfish, poultry, cattle, and pigs), and also from various human infections, making it a possible One Health indicator pathogen (Janda and Abbott, 2010; Figueras and Beaz-Hidalgo, 2015; Shen and Rodgers, 2018; Li *et al.*, 2020).

Previously, *Aeromonas* species has been observed to be resistant to a wild range of antimicrobial, which is attributed to indiscriminate use of antibiotics in aquaculture, plasmids and horizontal gene transfer. Multiple antibiotic resistance between *Aeromonas* species have been reported globally by different authors (Sen and Rodgers, 2017). Most Aeromonas spp are resistant to ampicillin, while third generation cephalosporin, fluroquinolone and aminoglycosides demonstrated excellent antimicrobial activity (Araujo *et al.*, 2015). *Aeromonas* isolates are intrinsic or chromosomally mediated resistant in cases of water related wound infections not responding to treatments with ampicilin, amoxicillin (Figueras and Beaz-Hidalgo, 2015). Although, ciprofloxacin and third generation cephalosporins are excellent antimicrobials in the treatment of *Aeromonas* associated infections and trimethoprin appears a viable option for the treatment of such infections (Sen and Rodgers, 2017).

Aeromonas spp have been implicated in both humans and animals and their importance to public health is a consequence of their high virulence and resistance to antimicrobials. Reviewed literature indicated that there is an increasing report in number of cases of gastroenteritis due to Aeromonas in Nigeria and worldwide, vectored by contaminated food and water. However, there is paucity of data on the occurrence of *Aeromonas* in piggery farms located in Ebonyi State Nigeria. The study detects the presence of *Aeromonas* spp in piggery farms and determines the antimicrobial resistant pattern of the isolates.

MATERIALS AND METHODS

Approval and consent for the study were obtained from the owners of the various pig farms. The study was conducted from March 2020 to June 2020 at the Microbiology Laboratory of Ebonyi State University Abakaliki, Nigeria.

Fifty (50) fresh Pig fecal samples were randomly collected from 25 different piggery farms from various parts of Abakaliki in Ebonyi State. All samples were collected aseptically using universal sterile container and immediately transported to the laboratory with ice packs and analyzed.

Aeromonas spp was isolated using standard microbiological procedures according to Cheesbrough (2006). Approximately one (1 ml) each of the samples was aseptically measured into test tubes containing 9 ml of sterile distilled water and shaken thoroughly for even distribution of organisms to make a stock. Thereafter, ten-fold serial dilution of the samples was carried out and subsequently inoculated on a freshly prepared nutrient agar plates and incubated at 37℃ for 24 hours. After incubation, some colonies were transferred to freshly prepared *Aeromonas* agar and allowed to grow for 24 hours. Growth were observed and representative colonies were picked, sub-cultured and subsequently identified using biochemical tests, that include; Fermentation of mannitol, catalase, coagulase, and thermostable DNase. Antibiotics Sensitivity testing was done on Muller Hinton Agar (Oxoid, UK) plates using standard disk diffusion method according to Clinical and Laboratory Standard Institute (2017). The antibiotic disks used include; imipenem (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), cefeprime (30 µg), meropenem (10 µg), tobramycin (10 µg) ceftazidime (30 µg) and amoxicillin clavulanic acid (30 µg). All the antibiotics disk were procured from Oxoid limited (Oxoid, UK). These antibiotics were chosen either because they are used in both medicine and human veterinary practice or as a result of previous studies with reports of microbial resistance to them. Colonies of confirmed *Aeromonas* isolates were collected using wire loop and were dispensed into test tubes containing 5 ml distilled water. The cell concentration was adjusted to 0.5 MacFarland standard and these were streaked on freshly prepared Mueller-Hinton agar plates.

The plates were allowed to stand for 15 minutes so that the cells will adapt to the environment of the medium. After this, the standard antibiotic disks were placed 15 mm apart on the plates and incubated at 30ºC for 24 hours and the zones of inhibition were measured according to CLSI criteria (2017)

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR = a/b (where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility). MARI of relative ratio >1 is shown to represent potential risk source of resistant strain from the environment (Raiz *et al*, 2011)

Statistical Analysis

Generated data was presented as mean±standard deviation which was used to analyze the data. Values of $P \leq 0.05$ were considered significant

RESULTS

The study revealed the presence of *Aeromonas spp* in the pig feacal samples from the study area The study revealed that mcrobial load of pig feacal samples from the study area ranged from $5.8\pm2.2x10^6$ CFU/ml to $22.0\pm9.6x10^6$ CFU/ml across the locations. It showed that samples collected from location designated **PG_6** recorded the highest microbial load ($22.0\pm9.6x10^6$), followed by samples from **PG_5** ($19.2\pm0.6x10^6$), while **PG_1** had the least microbial load ($5.8\pm2.2x10^6$)(Table 1).

Figure 1 shows the distribution of *Aeromonas* species among the samples collected from different locations. It revealed that samples collected from **PG_{18}** had the highest prevalence (10%), followed by samples from **PG_{10}** (8%), while samples from **PG_2**, **PG_6**, **PG_{14}**, **PG_{13}**, **PG_{16}** and **PG_{24}** had the least prevalence of (2%).

The study showed that the isolates exhibited varying degree of resistance against antibiotics used. The isolates showed 100% resistance to amoxicillin and cefuroxime followed by cefotaxine (90%), tobramycin (80%), ceftazidine (70%), and meropenem (60%) (Table 2). The isolates were more susceptible to imipenem (90%), followed by cefepime (60%) (Table 2)

The result of the multiple antibiotics resistant index among the *Aeromomnas* isolates showed that the isolates MAR index ranges from 0.4 to 0.9 with mean MARI value of 0.7 across all locations. The isolates with the highest MAR index includes isolates from **PG_{22}**, **PG_8**, **PG_{11}**, **PG_3**, **PG_{15}** and **PG_{11}** which exhibited 90% resistance to the tested antibiotics, while the least which recorded MARI index of 0.4 being resistant to 3/8 of the antibiotics was from **PG_{16}**

Table 1: Microbial load of piggery samples

Sample code	CFU/ml($\times 10^6$)
PG_1	5.8±2.2
PG_2	15.1±0.4
PG_3	10.2±4.2
PG_4	10.4±1.7
PG_5	19.2±0.6
PG_6	22.0±9.6
PG_7	15.4±8.2
PG_8	14.8±4.5
PG_9	14.4±3.9
PG_{10}	14.0±1.1
PG_{11}	14.8±5.6
PG_{12}	13.6±1.7
PG_{13}	13.4±7.1
PG_{14}	17.2±5.1
PG15	11.4±1.4
PG_{16}	11.2±6.2
PG_{17}	20.4±1.1
PG_{18}	14.0±4.5
PG_{19}	21.2±2.3
PG_{20}	8.0±1.7
PG_{21}	17.6±1.1
PG_{22}	13.3±1.3
PG_{23}	9.8±0.8
PG_{24}	12.4±1.1
PG_{25}	6.0±1.7

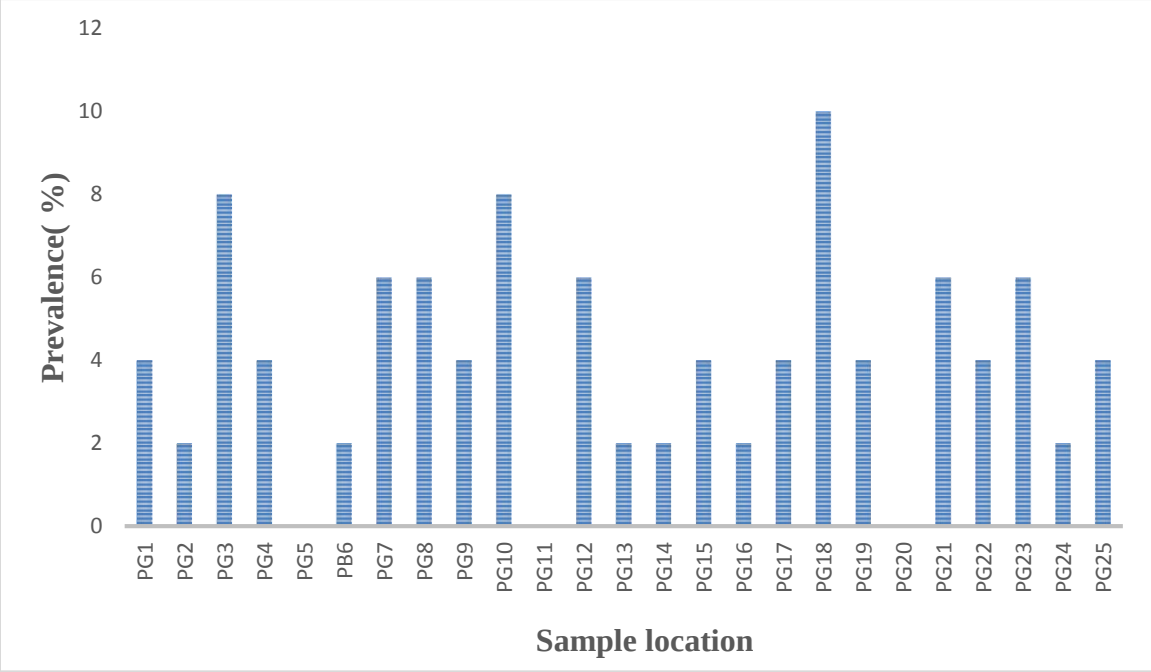

Figure 1: Prevalence of *Aeromonas* spp from different sample locations

Table 2: Antibiotics susceptibility ~~study~~ profile of *Aeromonas* spp against various antibiotics

Antibiotics	Resistance (%)	Intermediate (%)	Susceptible (%)
IPM	5(10)	0(0)	45(90)
CTX	45(90)	0(0)	5(10)
AMC	50(100)	0(0)	0(0)
CXM	50(100)	0(0)	0(0)
TOB	40(80)	0(0)	10(20)
CAZ	35(70)	0(0)	15(30)
FEP	15(30)	5(10)	30(60)
MEM	30(60)	0(0)	20(40)

Keys**:** IPM=Imipenem, CXM=Cefuroxime, CTX=Cefotaxine, FEP=Cefepime, MEM=Meropenem,
TOB=Tobramycin, CAZ=Ceftazidine, AMC=Amoxicillin

Table 3: Multiple antibiotics resistance index (MARI) of Aeromonas isolated from the different locations

LOCATION	MARI	ANTIBIOTICS
PG_{20}	0.5	CTX, AMC, CXM, MEM
PG_{24}	0.6	CTX AMC CXM TOB CAZ
PG_{22}	0.9	CTX AMC CXM TOB CAZ FEP MEM
PG_{8}	0.9	CTX AMC CXM TOB CAZ FEP MEM
PG_{19}	0.8	CTX AMC CXM TOB CAZ MEM
PG_{21}	0.8	CTX AMC CXM TOB CAZ MEM
PG_{16}	0.4	AMC CXM TOB
PG_{8}	0.8	IPM CTX AMC CXM TOB CAZ
		CTX AMC CXM
		CTX AMC CXM TOB CAZ MEM
PG_{16}	0.4	CTX AMC CXM MEM
PG_{18}	0.8	CTX AMC CXM TOB CAZ MEM
PG_{12}	0.5	CTX AMC CXM TOB CAZ
PG_{6}	0.8	CTX AMC CXM TOB CAZ FEP MEM
PG_{17}	0.6	CTX AMC CXM MEM
PG_{3}	0.9	CTX AMC CXM TOB CAZ MEM CTX AMC
PG_{24}	0.5	CXM TOB CAZ FEP MEM CTX AMC CXM
PG_{23}	0.8	TOB CAZ FEP MEM CTX AMC CXM
PG_{15}	0.8	MEM
PG_{11}	0.6	
PG_{2}	0.9	
Mean	0.7	

Key: IPM=Imipenem, CXM=Cefuroxime, CTX=Cefotaxine, FEP=Cefepime, MEM=Meropenem, TOB=Tobramycin, CAZ=Ceftazidine, AMC=Amoxicillin.

DISCUSSION

The findings of the study demonstrated that *Aeromonas* spp existed in high densities in pig faecal samples in the study area with microbial counts that ranges from $5.8\pm2.2\times10^6$ CFU/ml to $22.0\pm9.6\times10^6$ CFU/ml. The study further shows that the prevalence of *Aeromonas* spp among the different pig farms ranges from 2 to 10% The high microbial loads obtained in some of the sampled farms is an indication that pigs reared for human consumption in the area are highly contaminated posing a public health threat. The high contamination level could be as a result of poor hygiene in animal rearing and improper handling of pigs during the course of rearing them. The findings of this study correlates with study of Andelova *et al* (2006) who reported a prevalence of 8% of *Aeromonas spp* in pig droppings. However, the observation of the present study shows significant variation with the report of Grim *et al* (2014) who reported a prevalence of 22% to 31% of *Aeromonas* spp from pig droppings.

The findings of the study indicated varied susceptibility pattern of the *Aeromonas* spp isolated from various location of the study area against the different antibiotic used. The isolates exhibited 100% resistance to amoxicillin and cefuroxime followed by cefotaxine, while tobramycin, ceftazidine, meropenem, cefepime and others shows various degrees of resistances. The observation of this study is consistent with other works reported in Nigeria and other parts of the world (Isoken and Okoh, 2012; Usui *et al*., 2016; Chen *et al*., 2016; Deng *et al*., 2016; Edberg *et al*, 2007). Sen and Rodgers (2017) expounded that high antibiotic resistance could be attributed to the indiscriminate use of antibiotics in aquaculture, plasmids and horizontal gene transfer and the high demand for white meat globally.

Multiple antibiotic resistance among *Aeromonas* species is gradually becoming a global concern since several phenotypes of the organism has been associated with indiscriminate use of antibiotics in agriculture, hospital and environmental sources making the *Aeromonas* spp. an integral component for effective marker in monitoring antimicrobial resistance in both aquatic and agricultural settings (Usui *et al*., 2016) and also a possible indicator bacteria that allows for the dissemination of antibiotic resistance in an aquatic environment (Baron *et al*., 2017). The MAR index of the test isolates of *Aeromonas* spp examined in this study ranged from 0.4 to 0.9 with the mean MAR index of 0.7. The MARI value observed in this study is higher than the ≤ 0.2 recommended MAR index for bacteria that have not been exposed to indiscriminate and continuous use of antibiotics. Several other authors have reported similar high MARI in their studies Igbinosa *et al* (2012) reported high MARI index on bacteria associated with abattoir and aquaculture effluents, also Onuoha *et al* (2016) reported a high MARI among multiple drug resistant Gram negative and Gram-Positive bacteria isolated from abattoir waste and its receiving waters in Ebonyi State, Nigeria. Other studies indicated that *Aeromonas* spp has over the years displayed a consistent MDR across different sources such as hospital, water, livestock, poultry, aquaculture, food and environmental sources making them to be a widespread opportunistic pathogen (Fatih *et al*., 2007; Zhou *et al*., 2019; Marta *et al*., 2020; Ana and Maria, 2020). Furthermore in Nigeria generally, animal farms and fish ponds are located within human habitats thereby predisposing the human population to the risk of antimicrobial resistance genes (ARGs) within these areas. In their contribution, Ana and Maria (2020) identified the need for adequate monitoring of *Aeromonas* spp in water and food samples because there is an assumption that infections produced by the Aeromonads may constitute a great public health problem in the near future because of its diverse ecology and complex pathogenicity.

CONCLUSION

The study demonstrated that fecal samples obtained from pigs reared at different farms in Abakaliki contains high concentration of antimicrobial resistant species of *Aeromonas* with high MAR index. This is a serious problem to consumers who intend to sort for white meat and protein from pigs and as well to the treatment of infectious complications associated to multi drug resistance. The presence of *Aeromonas* specie in pig faecal samples poses great threat to the consumer's health. It is preeminent that measures should be taken to circumvent this situation.

REFERENCES

Ana, F. and Maria J. F. (2020). An Update on the Genus *Aeromonas*: Taxonomy, Epidemiology and Pathogenicity. *Microorganisms*, 8: 129.

Andelova, A., Porazilova, I. and Krejci, E. (2006). Correspondence. *Aeromonas* agar is useful selective medium for isolating aeromonads from faecal samples. *Journal of Medical Microbiology*, **55**:16-25.

Araújo, V. S, Pagliares, V.A., Queiroz, M. L. and Freitas-Almeida, A.C. (2015).Occurrence of *Staphylococcus* and enteropathogens in soft cheese commercialized in the city of Rio de Janeiro, Brazil. *Journal of Applied Microbiology*, 92(6):1172–1177.

Baron S, Granier S. A, Larvor E, Jouy E, Cineux M, Wilhelm A, Gassilloud B, Le Bouquin S, Kempf I and Chauvin, C (2017) . *Aeromonas* diversity and Antimicrobial Susceptibility in Freshwater—an attempt to set generic epidemiological cut-off values. *Frontier in Microbiology*. 8:503.

Cheesbrough, M (2006) District laboratory practice in tropical countries, Part 2. Cambridge University Press, Cambridge, UK. 23-78, 137-159.

Clinical and Laboratory Standard Institute (CLSI) (2017.). Performance Standard for antimicrobial Susceptibility Testing M02-A10. M07-A10 and M11-A8 27th edition

Cabié, A, Hope-Rapp, E, Beaucaire, G.; Hochedez, P, Nicolas, M and Olive, C. (2010). Bacteremia Caused by *Aeromonas hydrophila* Complex in the Caribbean Islands of Martinique and Guadeloupe. *American Journal of Tropical .Medicine Hygiene*, 83: 1123–1127

Chen, P.L, Lamy, B and Ko W.C (2016). *Aeromonas dhakensis*, an Increasingly Recognized Human Pathogen. *Frontier in Microbiology*. 7:793

Dascklov, H. (2013). The importance of *Aeromonas hydrophilia* in food safety. *Food control*, 17(6):474-483

Deng Y, Wu Y, Jiang L, Tan A, Zhang R and Luo, L (2016). Multi-Drug Resistance Mediated by Class 1 Integrons in *Aeromonas* Isolated from Farmed Freshwater Animals. *Frontiers in Microbiology*, 7:935.

Edberg, S. C., Browne, F. A. and Allen, M. J. (2007). Issues for microbial regulation: *Aeromonas* as a model. *Critical Review on Microbial infection*, 33:89-100.

Fatih M., Ayşenur K and Sadık D. (2007). Distribution and antibacterial drug resistance of *Aeromonas* spp. from fresh and brackish waters in Southern Turkey**.** *Annals of Microbiology*, 57 (3**):** 443-447.

Figueras, M.J and Beaz-Hidalgo, R (2015) *Aeromonas* infections in humans. In *Aeromonas*; Caister Academic Press: Norfolk, UK, pp. 65–108.

Gowda, T.K.G.M, Reddy, V.R.A.P, Devleesschauwer, B, Zade, N.N, Chaudhari, S.P, Khan, W.A, Shinde, S.Vand Patil, A.R (2015) Isolation and Seroprevalence of *Aeromonas* spp. Among Common Food Animals Slaughtered in Nagpur, Central India. *Foodborne Pathogenic disease*, 12: 626 – 630.

Grim, C. J., Kozlova, E.V and Ponnusamy, D (2014). Functional genomic characterization of virulence factors from necrotizing fasciitis-causing strains of *Aeromonas hydrophila*. *Applied Environmental Microbiology,* 80: 41-45.

Grim, C. J., Kozlova, E.V. and Sha, J. (2013). Characterization of Aeromonas hydrophila wound pathotypes by comparative genomic and functional analyses of virulence genes. *Microbiology of infection control agencies,* 4: 64-67

Isoken H. I and Okoh, A.I (2012). Antibiotic Susceptibility Profile of *Aeromonas* species isolated fromWastewater Treatment Plant. *The Scientific World Journal,* 2

Igbinosa , I.H, Beshiru, A and Igbinosa, E.O (2017). Antibiotic resistance profile of *Pseudomnas aeruginosa* isolated from aquaculture and abattoir environment in urban communities. *Asian Pacific Journal of Tropical Disease,* 7(1): 47 – 52.

Janda, J.M and Abbott, S.L (2010). The Genus *Aeromonas*: Taxonomy, Pathogenicity, and Infection. *Clinical microbiology review,* 23: 35–73.

Joseph, S. W and Carnahan A. M (2015). Update on the genus *Aeromonas*. *Journal of Antimicrobial control,* 66:218-223

Liao, K.C.; Yen, P.T and Liu, C (2010). Necrotizing fasciitis Caused by Inconspicuous Infection of *Aeromonas hydrophila* in an Immuno-compromised Host. J. Surg. Case Rep, 2.

Li, T, Raza, S.H.A, Yang, B, Sun, Y, Wang, G, Sun, W, Qian, A, Wang, C, Kang, Y and Shan, X. (2020) *Aeromonas veronii* infection in commercial freshwater fish: A Potential threat to hublic health *animals*, 10: 608.

Marta Z, Zbigniew J. M. and Piotr, P. (2020). Abundance and antibiotic resistance of *Aeromonas* isolated from the water of three carp ponds. *Veterinary Research Communications*, 44:9–18.

Neyts, K., Huys, G., Uyttendaele, M. J. and Debevere, J. (2014). Incidence and identification of mesophilic *Aeromonas* spp. from retail foods. *Applied Microbiology of life,* **31:**359-363.

Onuoha, S.C, Okafor, C.O., Aduo, B.C and Nwaka, F.C. (2016) Distribution of Antibiotic Resistant Bacteria from Abattoir Wastes and its Receiving Waters at Nkwo-Ezzamgbo, Ebonyi State, Nigeria, *World Journal of Medical Sciences*, 13 (4): 242-250

Riaz S, Faisal M and Hasnain S (2011). Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum β-lactamase (ESBL) producing *Escherichia coli and Klebsiella* spp. in Pakistan. *African Journal of Biotechnology,* 10(33): 6325- 6331.

Sen, K. and Rodgers, M. (2017). 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.

Shen, Y, Xu, C. Sun, Q, Schwarz, S., Ou, Y. Yang, L, Huang, Z, Eichhorn, I, Walsh, T.R and Wang, Y (2018). Prevalence and Genetic Analysis of mcr-3-Positive *Aeromonas* Species from Humans, Retail Meat, and Environmental Water Samples. *Antimicrobial Agents Chemotherapy,* 62: 1.

Usui M, Tagaki C, Fukuda A, Okubo T, Boonla C, Suzuki S, Seki K, TakadaH and Tamura Y (2016) Use of *Aeromonas* spp. as General Indicators of Antimicrobial Susceptibility among Bacteria in Aquatic Environments in Thailand. *Frontiers in Microbiology*. 7:710.

Zhou, Y, Li Y, Zheng N, Pingping Z, Biao K, Donghui Y and Jianrong, S. (2019). Taxonomy, virulence genes and antimicrobial resistance of *Aeromonas* isolated from extra-intestinal and and intestinal infections, *BMC infectious disease,* 19: 158