

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

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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 fecal 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 $5.8 \pm 2.2 \times 10^6$ CFU/mL to $22.0 \pm 9.6 \times 10^6$ CFU/mL across the locations. The isolates showed 100% resistance to amoxicillin and cefuroxime followed by cefotaxime (90 %), tobramycin (80 %), ceftazidime (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 Enterobacteriaceae. 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 wide range of antimicrobials, 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 ampicillin, amoxicillin (Figueras and Beaz-Hidalgo, 2015). Although, ciprofloxacin and third generation cephalosporins are excellent antimicrobials in the treatment of *Aeromonas* associated infections and trimethoprim 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°C 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), cefepime (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 30°C 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 faecal samples from the study area. The study revealed that microbial load of pig faecal samples from the study area ranged from $5.8 \pm 2.2 \times 10^6$ CFU/ml to $22.0 \pm 9.6 \times 10^6$ CFU/ml across the locations. It showed that samples collected from location designated **PG₆** recorded the highest

microbial load ($22.0 \pm 9.6 \times 10^6$), followed by samples from **PG₅** ($19.2 \pm 0.6 \times 10^6$), while **PG₁** had the least microbial load ($5.8 \pm 2.2 \times 10^6$) (Table 1).

Figure 1 shows the distribution of *Aeromonas* species among the samples collected from different locations. It revealed that samples collected from **PG₁₈** had the highest prevalence (10%), followed by samples from **PG₁₀** (8%), while samples from **PG₂**, **PG₆**, **PG₁₄**, **PG₁₃**, **PG₁₆** and **PG₂₄** 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 cefotaxime (90%), tobramycin (80%), ceftazidime (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 *Aeromonas* 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 **PG₂₂**, **PG₈**, **PG₁₁**, **PG₃**, **PG₁₅** and **PG₁₁** 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 **PG₁₆**.

Table 1: Microbial load of piggery samples

Sample code	CFU/ml($\times 10^6$)
PG ₁	5.8 $\pm$ 2.2
PG ₂	15.1 $\pm$ 0.4
PG ₃	10.2 $\pm$ 4.2
PG ₄	10.4 $\pm$ 1.7
PG ₅	19.2 $\pm$ 0.6
PG ₆	22.0 $\pm$ 9.6
PG ₇	15.4 $\pm$ 8.2
PG ₈	14.8 $\pm$ 4.5
PG ₉	14.4 $\pm$ 3.9
PG ₁₀	14.0 $\pm$ 1.1
PG ₁₁	14.8 $\pm$ 5.6
PG ₁₂	13.6 $\pm$ 1.7
PG ₁₃	13.4 $\pm$ 7.1
PG ₁₄	17.2 $\pm$ 5.1
PG ₁₅	11.4 $\pm$ 1.4
PG ₁₆	11.2 $\pm$ 6.2
PG ₁₇	20.4 $\pm$ 1.1
PG ₁₈	14.0 $\pm$ 4.5
PG ₁₉	21.2 $\pm$ 2.3
PG ₂₀	8.0 $\pm$ 1.7
PG ₂₁	17.6 $\pm$ 1.1
PG ₂₂	13.3 $\pm$ 1.3
PG ₂₃	9.8 $\pm$ 0.8
PG ₂₄	12.4 $\pm$ 1.1
PG ₂₅	6.0 $\pm$ 1.7

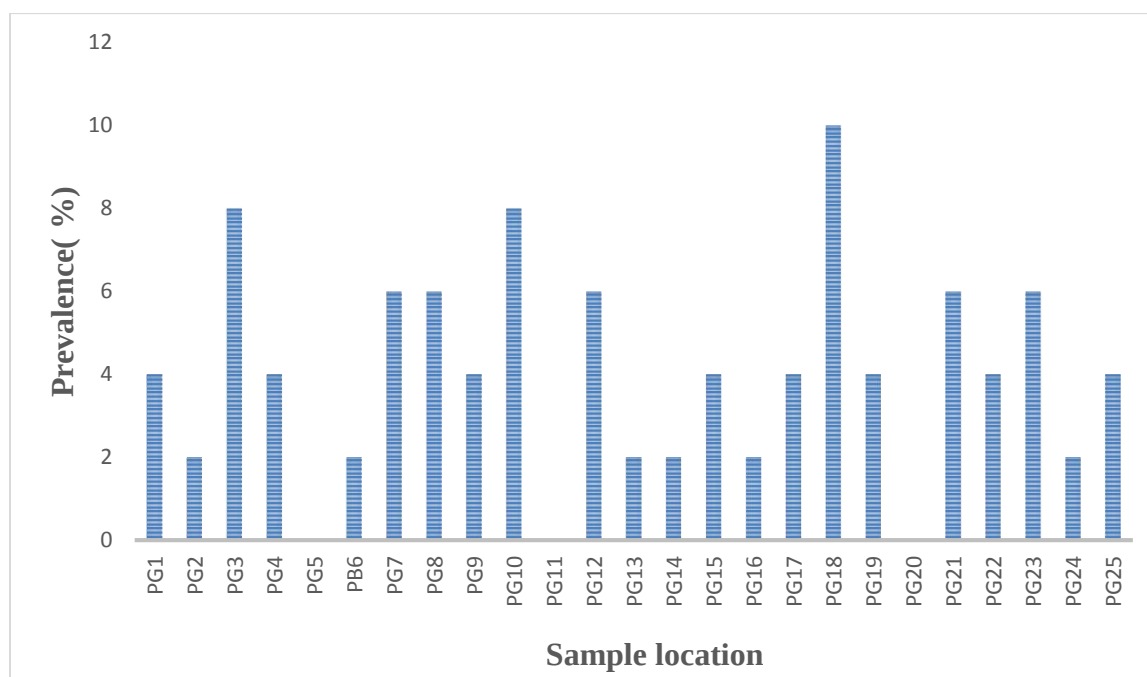
**Figure 1: Prevalence of *Aeromonas* spp from different sample locations**

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 $5.8 \pm 2.2 \times 10^6$ CFU/ml to $22.0 \pm 9.6 \times 10^6$ 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 cefotaxime, while tobramycin, ceftazidime, 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.

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