Original Research Article

Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Chicken: A Public Health Concern.

ABSTRACT

Infection of *Pseudomonas aeruginosa* has been responsible for economic losses in younger birds. This research was designed to report and document multidrug resistant *P. aeruginosa* recovered from chicken during infection. Sixty-seven freshly dead chicken were used in this research, the trachea, the liver, and the heart were targeted for *P. aeruginosa* isolation. The isolates were identified based on the cultural, morphological, and biochemical characteristics. Antimicrobial test was carried out on the pure isolates using the disc diffusion method. A total of 23 isolates of *P. aeruginosa* were recovered; 19 isolates were recovered from the trachea, 3 from the liver and 1 from the heart. All the isolates were showing green pigment on nutrient agar, they were all Gram-negative rods, motile, catalase and citrate positive. The result of the antibiotic susceptibility showed that ampicillin was most resisted with a resistance of 95.7% (22) while gentamicin was least resisted with 39.1% (9), and 69.5% (16) of the isolates were showing resistance to more than three antibiotics. The most encountered resistant pattern was AMP, TLY with 87%. The result from this study revealed that multidrug resistant *P. aeruginosa* in poultry may be emergingand serving as a reservoir for resistant *P. aeruginosa* gene.

Keywords: Antibiotics, Infection, *Pseudomonas aeruginosa,* Resistance

1. INTRODUCTION

Importance of*Pseudomonas* species in both veterinary and human clinical cannot be over emphasized.Members of this family is responsible for otitis in dogs, cats and may be found causing otitis media in human as in the case of *Pseudomonas otitis*. Of great clinical concern is *Pseudomonas aeruginosa* which has been incriminated in the poultry infections of young turkey and chicken [1]. As an opportunistic pathogen, *P. aeruginosa* thrive in moist environments and produces a water-soluble green pigment with a fruity odour. The nutritional requirement for its growth is relatively simple and gives it the opportunity to survive almost everywhere. They are generally found in the soil, on vegetation, feaces, water, and humid environments [2]. It may grow in almost distilled water, soap, and motor fuels. Even in extreme conditions, like in jet fuel, Pseudomonas toxins has been reported as a source of metal corrosion [2].

Pseudomonas aeruginosa infection may gain entrance into the poultry through various means which may include error in the management and industrial practices. The feed may also serve as a source of the infection [1, 3, 4]. In previous studies, many of the bacteria encountered in poultry were claimed to be bacteria of feed origin [5, 6].

P. aeruginosa is the most common pseudomonad causing infections. It has been reportedly causing the death of chicken with high virulence causing 50 -100% mortality in young chicken [3]. The disease of *Pseudomonas* induces significant economic losses to farmers by causing high mortality of newly hatched chickens and death of embryo. Generally, it is

considered to be an opportunistic organism that produces respiratory infections, septicemia and other forms when introduced into tissues of susceptible birds [3]. Though, in most veterinary textbooks Pseudomonas has never been recognized as a major threat to the poultry industry. However, the economic importance of this bacteria can never be underestimated because of the antibiotic resistance capacity and its possible ability to retain and transfer the gene [7].
Antibiotic resistance of *P. aeruginosa*to various antimicrobial agents was reported to be because of the impermeability of the membrane, the multi-drug efflux, and a chromosomal AmpC β-lactamase. Mutations may have result in upregulation of efflux or down regulation of permeability. The antibiotic resistance found in the clinical isolates of *P. aeruginosa* especially those from wound is of great importance to the public health. *Pseudomonas* has been recorded as one of the most common nosocomial bacteria often encounter during visit to the health care homes and has been reported as a multi resistant bacteria [8].
Moreover, there is little information about prevalence of and antibiotic resistance of *P. aeruginosa* in poultry, therefore, this study was designed to document the antibiotic resistance of *P. aeruginosa* isolated from chickenin Ekiti State, Southwestern Nigeria, relate the effect of the infection to poultry industry and the effect this infection could have on human population.

2. MATERIAL AND METHODS

Samples collection was based on the freshly dead chicken brought to the veterinary hospitals from twelve farms between January 2017 and June 2018. The samples (sixty-seven freshly dead chicken) were necropsied, the organs were collected and sent to the microbiology laboratory within one hour. Swabs were collected aseptically from the trachea, the heart, and the liver for bacteria isolation.

2.1. Bacteriology

The swabs collected were first activated in sterile buffered peptone water at 37^{o}C for five hours. A loop full was subsequently inoculated on MacConkey agar and nutrient agar (Biomark). The plates were inverted and incubated at 37°C for 24 hours in an incubator (Royalcare England. DNP 9022A).

2.2. Cultural and biochemical characterization

Pseudomonas aeruginosa isolates were selected based on the cultural characteristics, the morphological appearance, and biochemical tests. Some of the biochemical tests carried out were motility, catalase, oxidase, H_2S production, nitrate, urease, indole, methyl red, Voges-Proskauer and citrate use tests.

2.3. Antimicrobial drug sensitivity test

Pure culture of the identified *P. aeruginosa* isolates was tested against antimicrobial agents (in-vitro). The standard disk diffusion procedure was adopted. The organisms were standardized using McFarland standard at the absorbance of 450nm. The isolates were inoculated on Muller-Hinton agar using spread plate method, the plates were allowed to dry before the antibiotic disks were introduced. The antimicrobial agents tested were Ceftazidime (CAZ 30 µg), Cefuroxime (CRX 30 µg), Gentamicin (GEN 10 µg), Ciprofloxacin (CPR 5 µg), Ofloxacin (OFL 5 µg), Nitrofurantoin (NIT 300 µg), Ampicillin (AMP 10 µg), Amoxicillin (AMOX 30 µg), Enrofloxacin (ENRO10µg), Furazolidone (FUR 10 µg), Tylosin (TLY 10 µg) and Doxycycline (DOX10 µg). Following the application of antimicrobial discs, the plates were inverted and incubated at 37 °C for 24 h in an incubator (Royalcare England. DNP 9022A). The diameters of the zones of inhibition were measured (millimetres) and compared to internationally accepted standard to determine the susceptibility or resistance of the isolate.

3. RESULTS AND DISCUSSION

3.1 Sources of *Pseudomonas aeruginosa*

Twenty-three isolates of *P. aeruginosa*were recovered from the freshly dead chicken, this gave a 34.3% prevalence of the suspecting cases. A total of nineteen isolates were recovered from the trachea, three from the liver while one was recovered from the heart of the chicken. All isolates of *P. aeruginosa* showed green pigment that diffuse into the medium on nutrient agar. The colonies are circular, flat, and smooth to torch. The isolates grow well on MacConkey agar without fermenting lactose. The morphology of the isolates under microscope showed that they are Gram negative rods in cluster arrangement. The biochemical characteristics of the isolated *P. aeruginosa* showed that they are catalase and citrate positive as presented in table 1.
Isolating *P. aeruginosa* from the trachea of infected birds as observed in this study, is in line with the case report of Devriese et al. [9]. However, the report concludes that *P. aeruginosa* wasnot isolated from heart, blood, or liver tissue and from organs covered with fibrinous material [9]. Though, in this study, 3 isolates of *P. aeruginosa* were recovered from the liver and 1 isolate from the heart. Dinev et al. [4] reported that the location of the bacteria in the bird tissue may largely depend on the entry point and/or the ability of the bacteria to migrate. Isolating *P. aeruginosa* from the liver and heart therefore suggested the invasiveness of the bacteria from the entry point.

The source of *P. aeruginosa* in poultry birds has been identified as environmental [1]. In research carried out on the feed of poultry birds in 2015, Atere et al. [7] reported that *P. aeruginosa* were recovered from the poultry feed, this is an indication that the feed may have serve as a source of the bacteria in the poultry birds.
Thirteen percent of *P. aeruginosa* isolates were recovered from the liver of the freshly dead chicken, this result compares favorably with the reports of Elsayed et al. [1] where 12% of the *P. aeruginosa* isolates were recovered from the liver of the freshly dead chicken. This may be due to the motile nature of the bacteria having develop invasive mechanism which enable it to overcome the first line and non-specific defense in the birds.
The reason for the proliferation of this disease in bird flock has been attributed to the age of the birds, the immune system of the birds as well as the environmental factors which predispose the birds to the bacteria [10]. Niilo [10] concluded that the young and growing birds are always very susceptible to a very high risk.

Table1: Cultural, morphology and biochemical characteristics of *P. aeruginosa* isolates from chicken

Cultural characteristics	Gram reaction	Motility	Catalase	Oxidase	Indole	Methyl red	Voges-Proskauer	citrate	H_2S	Nitrate	Urase
Flat green colonies on nutrient agar.	- rod	+	+	+	-	-	-	+	-	+	+

Key: - Negative, + Positive

3.2Antibiotic resistance of *P. aeruginosa* isolates

The antibiogram showed that 95.7% (22) of the *P. aeruginosa* isolates were resistant to ampicillin while gentamicin had the least resistance with 39.1% (9) as presented in table 2. Sixteen (69.5%) of the isolates were showing resistance to more than three antibiotics, the most encountered antibiotic resistant pattern was AMP, TLY and AMP, TLY, AMOX, CAZ with 87% and 56.5% resistance respectively (table 3).

Table 2: Percentage resistance of *Pseudomonas aeruginosa* isolates from chicken

Isolates	CEF	CAZ	AMOX	OFL	TLY	CIP	ENRO	NIT	FURA	GEN	AMP
Trachea n=19	73.6 (14)	84.2 (16)	73.6 (14)	42.1 (8)	89.4 (17)	57.9 (11)	57.9 (11)	63.2 (12)	63.2 (12)	36.8 (7)	94.7 (18)
Liver n=3	66.7 (2)	100 (3)	100 (3)	66.7 (2)	100 (3)	33.3 (1)	100 (3)	66.7 (2)	66.7 (2)	33.3 (1)	100 (3)
Heart n=1	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	0 (0)	100 (1)	100 (1)	100 (1)	100 (1)
Total n=23	73.9 (17)	86.9 (20)	78.2 (18)	47.8 (11)	91.3 (21)	56.5 (13)	60.9 (14)	65.2 (15)	65.2 (15)	39.1 (9)	95.7 (22)

Key: Ampicillin (AMP), Amoxicillin (AMOX), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CPR), Enrofloxacin (ENRO), Doxycycline (DOX), Furazolidone (FURA), Gentamicin (GEN), Nitrofurantoin (NIT), Ceftazidime (CAZ) and Cefuroxime (CRX).

Table 3: Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from chicken

Pattern	**Number of isolates**	**Percentage %**
NIT, CIP, OFL	7	30.4
CEF, CAZ, CIP, GEN	4	17.4
AMOX, OFL, TLY, ENRO	7	30.4
AMP, TLY, AMOX, CAZ	13	56.5
AMP, ENRO, FURA	10	43.5
GEN, OFL,CIP	5	21.7
CEF, AMOX, FURA	10	43.5
CEF, CAZ, AMOX, OFL	4	17.4

ENRO, FURA, GEN, AMP	3	13.0
AMP, TLY	20	87.0
CEF, CAZ, AMOX, OFL, TLY, CIP, ENRO, FURA, GEN, AMP	1	04.3

The antibiotic resistance of *P. aeruginosa* in this study is similar to what have been previously reported in other bacteria of poultry origin. Atere [11] reported that 93.8% of salmonella isolates of poultry origin were resistant to ampicillin which is comparable to what is observed in this research where 95.7% of the *P. aeruginosa* were resistant. Gentamicin was found to have the best activity in this research and this finding conform to the observation of Elsayed *et al*. [1], whereregentamicin had the best activity against *P. aeruginosa* from poultry birds.

A study conducted on the antibiotic susceptibility of *Escherichia coli* of poultry origin [5] revealed that *E. coli* recovered from infected birds showed a very high resistance level, with 79.2% of the isolates showing resistant to amoxicillin. The observed resistance to amoxicillin is similar to the result obtained for *P. aeruginosa* in this research where 78.2 % of isolates of *P. aeruginosa* were resistant to the same antibiotic.

It was found that most of the *P. aeruginosa* had multiple resistance to the antibiotics used in this research, *P. aeruginosa* is resistant to various antimicrobial agents due to impermeability, multi-drug efflux, and a chromosomal AmpC β-lactamase as reported by Elsayed *et al*. [1].

Previous studies carried out on the antibiotic resistance of bacterial isolates of poultry origin was reportedly higher than isolates of feed or environmental sources [5, 6, 11]. In comparative research carried out on the antibiotic resistance of bacteria of poultry origin and bacteria of dog origin, Atere *et al*. [12] reported that bacterial isolates of poultry origin showed a higher antibiotic resistance compared to bacterial isolates of dog origin. It was reported that isolates of poultry had 89.6% resistance to tylosin while bacteria from dog had 40.7%, and 90.6% of the isolates from poultry resisted ampicillin while 74.1% was recorded for bacteria of dog origin. Atere *et al*. [12] concluded that the reason for high resistance observed in poultry may have resulted from farmers engagement in self-medication before clinical reports, which eventually leads to misuse of drug. Addition of antibiotics to poultry feed sub-optimally to enhance production may also have created enabling environment for bacteria to develop resistance. Van-den Bogaard *et al*. [13] also reported that crowding and poor sanitation may also increase the resistance in birds. Dashe *et al*. [14] reported that the antibiotic resistance often encounter in poultry could be attributed to the proliferation of fake or sub-standard drug in Nigeria. All these factors may have caused the increased antibiotic resistance observed in this study.

3.3Public Health concern

Though, the importance of *P. aeruginosa* in adult birds is often overlooked by veterinary clinicians, however the economic effect of this bacteria should not be underestimated in the poultry of both young andadult birds. The effect of the resistance gene carried by these bacteria can constitute a serious problem. It is very important to note that *P. aeruginosa* has unique ability to form biofilms [15], this ability makes it easy for these bacteria to cause infection while in community of other bacteria.The fact that *P. aeruginosa* can serveas a source of resistance gene in poultry birds or as reservoir deserves a great attention [7]. The multi-resistance gene may be transferred to more virulence bacterium and cause a lot of loss in the farm.

In human, *P. aeruginosa*has been responsible for a wide range of infections which include folliculitis, pneumonia, and others.*P. aeruginosa* of chicken origin also have the same potential of causing these infections. It is therefore, aserious public health issue if such multidrug resistance gene from chicken get its way into human population, hence this makes the infection of *P.aeruginosa* in chickena public health concern.

4. CONCLUSION

The idea that, “there is no economic importance attached to *P. aeruginosa* infection in poultry” should be erased. Importance of creating awareness among poultry farmers on the economic implication of *P. aeruginosa* infection cannot be overemphasized. Feed management, proper hygiene, proper use of antibiotics, especially clinical and laboratory test before administration of any antibiotics should critically be considered. Resistance gene might be reserved in these bacteria and eventually be transmitted to other bacteria or get its way into human population. Therefore, proper care is needed in the way of careful maintenance and administration of antibiotics in poultry.

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