



Multi-Antibiotic Resistance of Some Gram Negative Bacterial Isolates from Poultry Litters of Selected Farms in Benue State

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ABSTRACT

This study was aimed at isolating and identifying *Shigella*, *Salmonella* and *Escherichia coli* bacteria associated with poultry litter obtained from the University of Mkar Community, Adekaa, Gboko central and GRA Gboko. The study also determines the antibiotic sensitivity patterns of the associated bacteria. The bacteria were isolated and identified phenotypically following standard microbiological methods. The antibiotic sensitivity patterns of the isolated bacteria tested against Septrin, Chloranphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Pefloxacin, Tarivid and Streptomycin. *Shigella* and *Salmonella* were completely resistant to chloranphenicol, augmentin, pefloxacin, amoxicillin. *Shigella* were also resistant to all the antibiotics excepts Septrin and ciprofloxacin showed they are intermediate to the drugs. Percentage antibiotics susceptibility pattern of gram negative bacteria isolated from poultry litter showed all bacterial isolates (100%) were resistant to Chloranphenicol while most of the isolates were susceptible to Amoxicillin. All the bacterial isolates showed high level (10.2 MAR index) antibiotic resistance. The study found that there was close ($R = .516$) association between the isolates and antibiotics. There was also significant relationship ($X^2 = 5.871$) between isolates and occurrence at different sample sites. The study therefore recommends proper information dissemination to farmers and poultry feeds producers on dangers of antibiotic resistant strains, use of Probiotics in animal feeds to prevent gastro-intestinal infections and use of regulations to control poultry litter disposal.

Keywords: Multi-Antibiotics, Resistance, Gram Negative, Bacterial Isolates, Poultry Litter.

1. INTRODUCTION

Poultry is a major fast growing source of meat in the world today, representing a quarter of all the meat produced in 2000 [1]. The modern poultry industry can produce market ready broiler chickens in less than six weeks. This accomplishment is done through genetic selection, improved feeding and keen health management practices involving usage of antibiotics as therapeutic agents to treat bacterial diseases in intensive farming systems [2]. Acquired resistance against frequently used antibiotics have been observed since the introduction of these antimicrobial agents in human and veterinary medicine [3]. The use of antibiotics is a major factor in emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine [4]. The rise in antibiotics resistance have been reported in the past two decade [5] and antibiotic resistance still remains a global problem

today. In intensively reared food animals, antibiotics are administered for therapeutic purpose and as Antimicrobial growth promoters (AMGPs) to the whole flock rather than individuals [6]. Resistance to antibiotics can either be naturally occurring for a particular organism/drug combination or acquired resistance, where mis-use of anti-microbials results in a population being exposed to an environment in which organisms that have genes conferring resistance (either spontaneously mutated or through DNA transfer from other resistant cells) have been able to flourish and spread. Hence, the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their fecal flora contains a relatively high proportion of resistant bacteria [6]. Resistant strains from the poultry gut readily soil poultry carcasses and when consumed, they alter or affect human endogenous flora [7]. Gene transfer occurs majorly *in vivo* between gastrointestinal tract bacteria and pathogenic bacteria, as identical resistant genes are present in diverse bacterial species from different hosts [8].



In light of this, there is probability that most pathogenic bacteria that threaten human health may soon be resistant to all known antibiotics [9]. Certain antibiotics however are critical to human infections caused by multidrug resistant pathogens, or because alternative therapies are less effective or are associated with side effects [10]. The determination of the effectiveness of antimicrobial agents against specific pathogens-either human or animal source- is essential for proper therapy [11]. The development of resistance to antimicrobial drugs is a serious problem worldwide, which threatens the ability to treat infections in animals and humans [1]. Most resistance problem probably arose from inappropriate use of antibiotics which exposes infectious agents to sub therapeutic doses of antimicrobial agents [12]. Moreso, the use of antimicrobials in agriculture especially as growth promoters, chemotherapeutic and prophylactic agents in food animals bacteria are of public health implication [13, 14]. Thus, it became imperative to provide information on the multiple antibiotic resistant on some Gram-negative bacteria isolated from poultry litter from selected farms in Gboko metropolis.

2. MATERIALS AND METHODS

Sample Collection

Four hundred and eighty (480) samples from GRA Gboko, Central Gboko, Adekaa and University of Mkar community settlement area were freshly collected from the month of June to August 2011, at time interval of 3-4 hours between 8: am and 5: pm in sterile polypropylene containers. That was morning afternoon and evening and transported to the laboratory within 30 minutes for analysis.

Isolation and Identification of Bacteria

Isolation and identification of bacteria were done based on their morphological, Gram staining, cultural, and biochemical properties described by Chessbrough, [15]. representatives of the different colonies were selected according to their morphological characteristics and purified by successive sub culturing on *Salmonella-Shigella* agar and identified phenotypically based on standard methods [16, 17].

Salmonella-Shigella and Mackonkey Agar plate were used to differentiate among Gram-negative bacteria. *E. coli* bacteria ferment the lactose in the media; resulting in bacterial growth with a pink color (they do not produced any hydrogen sulfide). Member of the genus *Salmonella* does not ferment lactose, but do produce hydrogen sulfide gas, so the resulting colonies were colorless as describe by [18].

Gram staining

Gram staining was carried out as described by [19] to identify the gram negative bacteria.

Oxidase Test

Oxidase test was performed as described by [15].

Antibiotics Disk

The bacterial isolates were tested for resistance to 10 antibiotics produced by MAXIDISC (Maxi care medical Laboratories Ltd., Nigeria). These were: Septrin (30 µg), Chloranphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxicillin (10 µg), Augmentin (30 µg), Gentamycin (10 µg), Pefloxacin streptomycin (30 µg), Tarivid (10 µg) and Streptomycin (30µg). This testing was performed using the standard disc diffusion method [21]. The antibiotics susceptibility pattern of the isolates was interpreted using Progressive Diagnostics Manufacturers (PDM) Interpretative Chart.

Antibiotic Sensitivity Testing

The test was carried out as described by [11].

Measurement of Zone of Inhibition

The metric ruler was placed across zones of inhibition, at the widest diameter, and measured from one edge of the zone to the other edge in accordance to [12]. Zone diameters were reported in millimeters, as 'S' (sensitive), 'R' (resistant), or 'I' (intermediate) as describe by [12].

Sensitivity

A clear, "halo" (technically known as a "plaque or zone of inhibition) that appeared around the antibiotic disk, indicating an absence of bacteria as reported by [12].

Intermediate

Somewhat cloudy plaque appearance indicates not all the bacteria in the area around the disk were killed i.e using the method discuss by [22].

Resistant

In this case, the antibiotic disk would have no discernable plaque around it, [1].



Statistical Analysis

Regression analysis was used to estimate overall sensitivity and resistant pattern similarities of the bacterial resistance using their zones of inhibition [1]. Chi-square was also employed to test the level of significance between the isolates and the sample sites using Predictive Arithmetic Software (PASW).

3. RESULTS

Table 1 shows antibiotic susceptibility pattern of some Gram negative bacteria isolated from poultry litter. The result showed that *E. coli* had the highest rate of resistance with 0.8 mar index, followed by *Salmonella* (0.6) and *Shigella* (0.3). Moreso, *Shigella* had highest number of intermediate with five (5), while *E. coli* and *Salmonella* two (2) respectively. Furthermore, *Salmonella* was susceptible to (CPX and AM) while *E. coli* had none susceptibility response as also shown in table 1.

4. DISCUSSION

The rise in antibiotics resistance had been reported in the past two decade [5] and antibiotic resistance still remains a global problem today. In intensively reared food animals, antibiotics are administered for therapeutic purpose and as Antimicrobial growth promoters (AMGPs) to the whole flock rather than individuals [6]. Resistance to antibiotics can either be naturally occurring for a particular organism/drug combination or acquired resistance, where mis-use of anti-microbials results in a population being exposed to an environment in which organisms that have genes conferring resistance (either spontaneously mutated or through DNA transfer from other resistant cells) have been able to flourish and spread. High level of antibiotic resistance was observed in this study with Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Pefloxacin, Tarivid and Streptomycin to mention but just few. From the four sampling sites about three genus of Gram negative bacteria were isolated; these include *Shigella*, *Salmonella* and *Escherichia coli*. These organisms are of public health importance [14]. They all showed multiple resistance to above mentioned antimicrobial agents. However, the high level of *E. coli* resistance to tested antibiotic seems to correspond with the report of [23]. This may be attributed to constant contact between feed, poultry birds and fecal dropping as also reported by [24]. Most of the isolated bacteria like *E. coli* and *Salmonella* showed high level of resistance more than *Shigella*, and they constitute normal flora of the intestinal tract of poultry as reported by [25]. The bacterial isolates showed high level of antibiotic resistance against all used antibiotics. The result was in agreement with [26, 27] who reported that the abuse and misuse of antimicrobial agents for growth promotion and prevention of diseases has impressed a selective pressure that

causes discovery of more resistant bacteria. This is true with the bacteria associated with poultry litter in this study. Thus, the antibiotic selection pressure for resistance by bacteria in poultry is high and as a result their fecal floor contains higher proportion of resistant bacteria [6]. The Multiple Antibiotic Resistance (MAR) index is defined as a/b where 'a' represents the number of antibiotics to which the particular isolate is resistant and 'b' the number of antibiotics to which the isolate is exposed [28]. MAR index values higher than 0.2 were considered to have originated from high-risk sources where antibiotics are often used. MAR index values of less than or equal to 0.2-4.5 indicates strain originated from sources where antibiotics are seldom or never use. Statistical evaluation estimate overall similarities of the bacterial resistance using their zones of inhibition [1]. Conclusively, the present results provide evidence that poultry litter can serve as an environmental reservoir of multiple antibiotics resistant bacteria and hence as potential route for the entry of multidrug resistant zoonotic pathogens into human population. This have very important implications for human health, as multidrug resistant infections are difficult to treat and often requires expensive antibiotics and long term therapy. This can substantially increase the cost of treatment and even mortality.

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**Table 1: Antibiotic Susceptibility Pattern of Some Gram Negative Bacteria Isolated from Poultry Litter**

Isolate	AB										MAR INDEX
	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	
<i>Shigella</i>	R	R	I	R	S	I	I	I	I	S	0.3
<i>Salmonella</i>	R	R	I	S	S	R	I	R	R	R	0.6
<i>E. coli</i>	I	R	R	I	R	R	R	R	R	R	0.8

Key

SXT=Septrin, CH=Chloranphenicol SP= Sparfloxacin, CPX= Ciprofloxacin, AM = Amoxicillin, AU Augmentin, CN = Gentamycin, PEF = Pefloxacin, OFX = Tarivid S=Streptomycin. R= resistant, I = intermediate, S= susceptibility, AB=Antibiotics.

Table 2 presents percentage of Antibiotic susceptibility pattern of some Gram negative bacteria isolated from poultry litter. The results revealed that *Shigella* responded totally (100%) to CH. with 33.3% response to CP, CPX, AM, and CN. The organism had 66.7% response to SXT, AU, PEF, OFX and S. The table also shows that *Salmonella* had 33.3% response to SXT, CPX, PEF and OFX, 66.7% response to CP and CPX. Also observed was zero response to CH and AM. *E. coli* further showed 33.3% resistance to SXT and CPX, 66.7% to AM and zero to CH, CP, AU, CN, PEF, and S. Regression analysis also showed strong association ($R=.516$) between antibacterial resistivity and isolates.

Table 2: Percentage of Antibiotic Susceptibility Pattern of Some Gram Negative Bacteria Isolated from Poultry Litter

Isolate	AB									
	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
<i>Shigella</i>	67.7	100	33.3	33.3	33.3	66.7	33.3	66.7	66.7	66.7
<i>Salmonella</i>	33.3	0	66.7	33.3	0	33.3	66.7	33.3	33.3	0
<i>E. coli</i>	33.3	0	0	33.3	66.7	0	0	0	0	0

$R= .516$ $df=1$

Key

SXT = Septrin, CH=Chloranphenicol, SP=Sparfloxacin, CPX= Ciprofloxacin, AM = Amoxicillin, AU Augmentin, CN = Gentamycin, PEF = Pefloxacin, OFX = Tarivid and S=Streptomycin, AB= Antibiotics.

Table 3 shows bacteria isolated and their occurrence at different sites. The result revealed that GRA Gboko recorded highest with percentage (28.8%), followed by Adekaa (25.6%) and University of Mkar community (24.8%), while Gboko central was least with (20.9%). Chi-square results also showed a significant relationship ($X^2 = 5.871$) between occurrence of bacteria and sample site.

Table 3: Bacteria Isolated and their Occurrence at Different Sites

Sample Site	<i>Shigella</i> (%)	<i>Salmonella</i> (%)	<i>E. coli</i> (%)	Total Occurrence (%)
UMC	29(22)	56(24.5)	68(26)	153(25)
Adekaa	35(27)	67(29)	56(22)	158(26)
Gbokocentral	28(21)	40(17.5)	61(24)	129(21)
GRA Gboko	40(30)	66(29)	72(28)	178(29)
Total	132	229	257	618

$X^2 = 5.871$ $df = 6$ $P > 0.05$

Key: UMC = University of Mkar Community