Multiple Antibiotic Resistance among *Escherichia coli* Isolated from Selected Abattoirs in Northwestern Nigeria

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Abstract: *Escherichia coli* is one of the major contaminants in the abattoir because of its frequent association with both living and cattle carcasses. It is used as indicator for both contamination and prevalence of antibiotic resistance. Samples were collected from water, effluent and swabs of various surfaces from selected abattoirs in northwestern Nigeria. They were analyzed using microbiological techniques for isolation of *E. coli.* Fifty of these bacteria were randomly selected and tested against nine selected antibiotics: amoxicillin-clavulanic acid (30 µg), cefoxitin (30 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), sulfonamides and trimethroprim (25 µg), chloramphenicol (30 µg), vancomycin (30 µg) and erythromycin (15 µg)to determine their level of resistance to each of the antibiotics using disc diffusion method. The results showed highest resistance of *E. coli* isolates to vancomycin (92%), followed by amoxicillin-clavulanic acid (76%) and erythromycin (76%). None of the isolates was resistant to gentamicin and ciprofloxacin. However, resistance against chloramphenicol (8%), sulfonamides and trimethroprim (16%), and cefoxitin(24%) were low. Multiple antibiotic resistant index (MARI) was determined and 46 (92%) of the isolates were found to be multi-drug resistant. Indiscriminate use of antibiotics for treatment, as growth promoter in animal foods and poor hygiene practices could be responsible for this level of resistance. The high resistant *E. coli* could be a significant threat to public health due to the risk of transferring the bacteria into food chain hence, monitoring antimicrobial resistance and virulence is indispensable.

Keywords: Abattoir, Antibiotic resistance, *E. coli*, Isolation.

INTRODUCTION

Cattle are globally known to be reservoir of *E. coli* as commensals which are often without pathogenic effects to the host animals but their detection in foods indicates poor hygiene during production or processing which can compromise the health of the consumers (Atnafie *et al*., 2017). The few pathogenic species including shiga toxin-producing *E. coli* O157:H7 can constitute significant threat to public health such as abdominal pain, bloody diarrhoea, haemolytic colitis, and haemolytic uremic syndrome (HUS) therefore, monitoring their antimicrobial resistance and virulence cannot be overemphasized (Bok *et al*., 2015). These bacteria serve as indicator for quality and faecal contamination in food industries, and also in assessing the prevalence of antimicrobial resistance (Pissetie*t al*., 2016; Ariel *et al*., 2020). Enteric food-borne pathogens are known to be shed into the environment and their persistence in same environment may contaminate equipment, utensils, floors, walls and other surfaces (Park *et al*., 2014). This is made possible via diverse vehicles and activities such as unintentional deposition of faeces by infected or carrier livestock in the farm and slaughterhouse, application of raw or inadequately composted manure (Park *et al*., 2013).

In Medical sciences, antibiotics are meant to enhance treatment of infectious diseases, reduce morbidity and mortality (Caruso, 2018) unfortunately, different bacterial species began showing resistance to the potentials of these antibiotics under selective pressure, compromising the efficacy via different mechanisms (Mivey and Simor, 2009; Fair and Tor, 2014; WHO, 2015).

Antibiotic resistance has been a global public health threat in medical sciences and public health practice, challenging the control of infectious diseases and has deterred the progress on health outcomes, increasing cost of infectious disease treatment on societies (Leung *et al*., 2011; Capita and Alonso-Calleja, 2013). It was reported recently that antimicrobial resistant

bacteria of food-borne and animal-origin constitute burden to human health (WHO, 2017).

Various studies have revealed a great variability of antimicrobial resistance among isolates most especially those associated with bovine carcasses (Fontcuberta *et al*., 2016; Loiko *et al*., 2016; Murutu *et al*., 2016).

Emergence of multidrug-resistant bacteria is associated with livestock species including food animals, promoting the spread to humans and environment (Caruso, 2018). About 75-90% of antibiotics administered to food animals are not metabolized and are mostly excreted into the environment which could become a reservoir of resistance genes in the community (Marshall and Levy, 2011; ManyiLoh *et al*., 2018).

It is reported that more strains of pathogens have become antibiotic resistant to a number of antibiotics and chemotherapeutic agents, high rate of bacterial resistance to antibiotics and indiscriminate use has shown a worldwide trend towards the emergence of multi-resistance, causing challenges in treatment of diseases that might lead to outbreaks and increase in mortality rate (Nagai *et al*., 2016).This mortality rate has been reported globally including about 700,000 deaths by O'neill (2016), who also predicted that in 2050, over 10 million deaths would be recorded annually.

About 75-90% of antibiotics administered to food animals are not metabolized and are mostly excreted into the environment which could become a reservoir of resistance genes in the community (Marshall and Levy, 2011; ManyiLoh *et al*., 2018).The indiscriminate use of antibiotics in many countries potentially promotes the emergence and transmission of antibiotic resistant bacteria and resistance genes in various environment (Davies and Davies, 2010). The aim of this research was to determine the multiple antibiotic resistance of *E. coli* isolated from selected abattoirs in northwestern Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in the main abattoirs located in each of the four randomly selected states within North-western geopolitical zone of Nigeria namely: Zango Shanu Abattor located in Zaria, Kaduna State, Maiyanka abattoir in Kano, Kano state, Gusau Ultra-modern Abattoir in Zamfara State, and Dutse Ultra-modern Abattoir in Jigawa State as shown in Fig 1 below.

Figure 1: Map of North-Western Nigeria Showing Abattoir Locations
(Adapted from the administrative map of Nigeria/Fieldwork, 2014)

Study Design

A cross sectional study design was employed whereby a simple random sampling of water, effluent, and surface swab samples from each of the selected abattoirs were collected and analyzed.

Collection of Samples

Samples were collected from water used for washing carcasses, effluent generated during processing of carcasses and various surfaces including carcass hides, meat, walls, butchers' hand, knives, and floor of the abattoirs. The water samples were collected by dipping 250ml capacity of conical flask into the water-containers at a depth of about 5-10cm. Sterile swab sticks were used for sample collection from surfaces. Effluents (about 250ml) were collected from the top-, mid-, and down- stream of the channels. Approximately 192 samples were collected in each abattoir and a total number of 768 samples were collected from all the abattoirs during the period of study.

Samples from Zaria and Gusau abattoirs were conveyed on ice-parkto Microbiology postgraduate laboratory in Ahmadu Bello University, Zaria, Kaduna state while those from Kano and Dutse abattoirs were taken to Biology laboratory of Federal University Dutse, Jigawa state for microbial analyses.

Isolation of *Escherichia coli*

The isolation of *E. coli* involved inoculation of samples on eosin methylene blue agar and incubated at 37^0C for a maximum period of 48 hours. Green metallic sheen appearance on the agar indicated positive result for *E. coli*. Pure colonies were further identified using recommended conventional biochemical tests: catalase, oxidase, and motility (Tiwari *et al*., 2009; Cheesebrough, 2010) before the use of Microgen^{TM} GNA-ID System for biochemical identification following the manufacturer's protocols.

Antimicrobial susceptibility test

The bacteria were tested against the selected antibiotics based on guidelines of CLSI (2013) using disk diffusion method (Bauer-Kirby, 1996).The selected antibiotics based on CLSI (2013) guide included: amoxicillin-clavulanic acid (AUG: 30 µg), cefoxitin (FOX: 30 µg), gentamicin (GTN: 10 µg), tetracycline (TET: 30 µg), ciprofloxacin (CIP: 5 µg), sulfonamides and trimethroprim (STX: 25 µg), chloramphenicol (CHL: 30 µg), vancomycin (VAN: 30 µg) and erythromycin (ERY: 15 µg).Bacterial suspension was made from overnight culture and was standardized using McFarland turbidity of 0.5 after which a loopful of the bacterial suspension was streaked uniformly all over the surface of sterile Mueller Hinton Agar (MHA) after which each commercially prepared antibiotic single disc (Oxoid Ltd, England) was placed on the surface of the inoculated plates using a sterile forceps. The plates were incubated at 37^0C for 18 hours except for vancomycin after which the diameter of each zone of inhibitions formed against respective antibiotics were measured in millimeters. The cleared zone diameter of each disc was interpreted as susceptible, intermediate, or resistant using the recommended zone diameter and interpretative standards for Enterobacteriaceae (CLSI, 2013).

Multiple antibiotic resistant index (MARI) was determined for each isolate. Those with MARI > 0.2 were considered multi-drug resistant (Subramani and Vignesh, 2012; Tadesse, 2012).

RESULTS

Isolation of *Escherichia coli*

The results of characterization of *E. coli* isolated in this study is as shown in Table 1. Based on the manufacturer's guidelines, those positive for indole, lactose, mannitol, glucose, motility, lysine, ortho-nitrophenol and negative for H_2S oxidase, urease, citrate, and or Voges proskaeur tests were confirmed to be *E. coli* among others.

Table 1: Biochemical characterization of *Escherichia coli*

Isolate	GR	Lysine	Ornitine	H_2S	Glucose	Mannitol	Xylose	ONPG	Indole	Urease	VP	Citrate	TDA	Confirmed Bacteria
Z 53	-	+	+	-	+	+	+	+	+	-	-	-	-	*Escherichia coli*

Z 53: Isolate; GR: Gram's reaction; ONPG: ortho-nitrophenol; VP: Vogesproskaeur; TDA: Tryptophan Deaminase

Antibiotic Sensitivity of *Escherichia coli* isolated

The results of antibiotic sensitivity obtained for *Escherichia coli* as shown in Table 2 indicated that almost all the *E. coli* isolates were resistant to VAN (92%) and more than half of the population were resistant to AUG (76%), ERY (76%) and TET(60%). None of the bacteria was resistant to GNT and CIP. However, resistance against CHL (8%), SXT (16%), and FOX (24%) (Table 2) were relatively low. These imply that most of the *E. coli* isolates were susceptible to each of the antibiotics though at different levels. Some were highly susceptible to CIP 48 (96%), SXT and CHL 42 (84%), FOX 36 (72%) and low rate of susceptibility was recorded against ERY 2 (4%), to GNT and VAN 4 (8%) and AUG 8 (16%).

Table 2: Antibiotic susceptibility of some *E. coli* isolated from the abattoirs

Antibiotic	***E. coli* n=50 (%)**		
	Susceptibility	**Intermediate**	**Resistance**
AUG	8(16)	4(8)	38(76)
FOX	36(72)	2(4)	12(24)
GNT	50(100)	0	0
TET	20(40)	0	30(60)
CIP	48(96)	2(4)	0
SXT	42(84)	0	8(16)
CHL	42(84)	4(8)	4(8)
VAN	4(8)	0	46(92)
ERY	2(4)	10(20)	38(76)

S: susceptibility, I: intermediate, R: resistance AUG: amoxicillin/clavulanic acid, TET: tetracycline, FOX: cefoxitin, CHL: chloramphenicol, SXT: sulfamethoxazole, CIP: ciprofloxacin, VAN: vancomycin, GNT: gentamicin, ERY: erythromycin

Multiple Antibiotic Resistance

The multiple antibiotic resistance pattern of the selected 50 isolates were identified and classified based on the Multiple Antibiotic Resistance Index (MARI) and arbitrary value of risk contamination of 0.2 adapted from International Expert Proposal for Interim Standard (Krumperman, 1983; Magiorakos, 2012; Tadesse, 2012). Forty-six (92%) of the isolates were found to be resistant to minimum of three classes of antibiotics hence, they are considered multi-drug resistant whereas, 4(8%) of the isolates had MARI $\leq$ 0.2 and are considered non-multiple antibiotic resistant (Table 3)

A total of sixteen *E. coli*(isolated from hides, floor and effluent in Gusau abattoir, floor and butchers' hands in Dutse abattoir, and effluent from Kano abattoir) were found to be resistant to at least 3 antibiotics of two different sets: AUG, VAN,ERY and AUG, FOX, VAN.

Table 3: Multiple Antibiotic Resistance Pattern of the *Escherichia coli*

Antibiotic resistance pattern	*E. coli n=50*	MARI
AUG, VAN, ERY	14	0.3
AUG, FOX, VAN	2	0.3
AUG, VAN, ERY, TET	12	0.4
ERY, TET, SXT, CHL	2	0.4
AUG,VAN, ERY, CHL	2	0.4
AUG, VAN, FOX, ERY	2	0.4
AUG,VAN, ERY, TET, SXT	2	0.5
AUG,VAN, ERY, TET, FOX	6	0.5
AUG, VAN, ERY,TET, FOX, CHL	2	0.7
AUG, VAN, ERY,TET, FOX, SXT, CHL	2	0.8
	46(92%)	

MARI: Multiple Antibiotic Resistance Index; AUG: amoxicillin/clavulanic acid;TET: tetracycline; FOX: cefoxitin; CHL: chloramphenicol; SXT: sulfamethoxazole;CIP: ciprofloxacin; VAN: vancomycin; GNT: gentamicin; ERY: erythromycin

It was observed that *E. coli* among others were resistant to antibiotics of four different combinations including AUG, VAN, ERY, TET; AUG, VAN, ERY, CHL; ERY, TET, STX, CHL and AUG, VAN, FOX, ERY. Twelve of the *E. coli* isolates were resistant to the first set of 4 antibiotics comprising AUG, VAN, ERY, TET and were isolated from cattle hides, butchers' hands, knives and floors across all the abattoirs, the other six *E. coli* were resistant to AUG, VAN, ERY, CHL from wall in Zaria abattoir; ERY TET, STX, CHL from butchers hands in Zaria and Kano abattoir and AUG, VAN, FOX, ERY from Dutse and Kano abattoir effluents respectively.

It was also found that six of the bacterial isolates were resistant against a set of 5 antibiotic combinations**:** AUG, VAN, ERY, TET, SXT (butcher's hand and meat from Zaria, meat, floor and hides from Kano); and two against a combination of AUG, VAN, ERY,TET, FOX (knife from Gusau, effluent from Kano). Two *E. coli* were found to be resistant against one set of 6 antibiotic combinations: AUG, VAN, ERY, TET, FOX, CHL (floor and carcass hide from Zaria).Two *E. coli* isolates were resistant to a set of 7 antibiotics; AUG, VAN, ERY, TET, FOX, SXT, CHL (meat and effluent from Kano).

DISCUSSION

In this study, *E. coli* was frequently isolated from various environmental sources in the abattoirs. Their presence could be of public health importance because of the tendency to harbor resistance genes and the risk of introducing into food chain (Ariel *et al*., 2020). It is an important indicator which can be used as a gauge for hygienic practices, and its presence on hands of abattoir workers, carcass surfaces, knives and other surfaces could be related to hygiene practices in abattoirs, and might lead to contamination with enteric bacteria during evisceration, causing occupational diseases which can be transmissible to humans (Wheatley *et al*., 2010; Shamsul *et al*., 2016; Ariel *et al*., 2020).

Bacterial resistance to antibiotics is known globally to be in high rate and could result in a trend toward emergence of multi-resistant (Nagai *et al*., 2016). All isolates tested showed no resistance to GNT and CIP and nearly all isolates showed no resistance to CHL. This is similar to findings of Ariel *et al*. (2020) in a slaughterhouse in Brazil, Ahaduzzaman *et al*. (2014) in hospitals and slaughterhouses, and Unambra-Oparah *et al.* (2012) in Nsukka municipal abattoir.

This implies that these bacteria may have not develop resistance genes yet, and probably they are not being frequently and or indiscriminately used during cattle raring. The low rate of resistance (high susceptibility) implies that these drugs could be effective in treating *E. coli* infections.

Highest resistance of *E. coli* to VAN in this study could be associated with development of intrinsic resistance probably acquired due to mutation in chromosomal DNA or by acquiring new resistance genes on transferable DNA segments (Shehabi *et al*., 2006)

Some of the selected antibiotics: AUG, SXT, TET, CHL and ERY satisfied the guidelines for the responsible and prudent use of antimicrobial agents in veterinary medicine (Rajesh, 2014).The percentage resistance of *E. coli* to chloramphenicol was low, however, the use is not approved in food animals but its persistent resistance in *E. coli* in the United States has been observed by certain authors including US Food and Drug Administration (2010). Most of the few chloramphenicol-resistant *E. coli* isolates are concurrently resistant to tetracycline. It inhibits protein synthesis by blocking peptide bond formation and binds to the 50S ribosome subunit, interfering with the binding of the aminoacyl moiety of the aminoacyl-tRNA. Over the past years, aminoglycosides (GNT) resistance has increased among Enterobacteriaceae rods as shown in the results obtained by Ojdana *et al*. (2018) among others in which resistance to aminoglycosides was found in 79.5% of the isolates. In contrast, results in this study have shown no resistance by the *E. coli* probably due to the fact that most samples considered in this study were of environmental source.

The high susceptibility rate (>70%) of the tested bacteria to selected antibiotics in this study might be due to the fact that the bacteria were community acquired. More so, it could be that most of the antibiotics are not commonly used in cattle farms (where they are reared) and abattoirs in question. Indiscriminate use of antimicrobials due to inappropriate prescribing and dispensing, absence of legislation rules in the use and poor enforcement(Togoobaatar *et al*., 2010), causing disease outbreaks and increase in mortality rate (Nagai *et al*., 2016). This could result in transmission of antibiotic resistant genes within pathogenic strains (Shitandi and Sternesjo, 2004). Despite the lower rate of resistance obtained, the traits might be present in the bacteria, usually coded and carried on chromosomes, plasmids, integrons and could be easily transferred among bacterial isolates (Rychlik *et al*., 2006).

The *E. coli* had 92% multiple drug antibiotic resistance which indicated high risk contamination (Krumperman, 1983). Higher index values of some bacteria (>0.2) suggested high and indiscriminate use of antibiotics in their environment of origin (Tambekar*et al*., 2006). This is applicable to the cattle farm where the cattle to be slaughtered in the abattoirs in question were reared. Multiple antibiotic resistance index profile showed that majority of the *E. coli* were resistant to a combination of 3 and 4 antibiotics while few were resistant to 5, 6, and 7 combinations. This is similar to results obtained by Falodun and Ajala (2018) from an abattoir in Ibadan, Nigeria. This might be due to inappropriate and frequent use of these antibiotics (AUG, VAN, ERY,TET, FOX, SXT, CHL)as growth promoters and prophylaxis in cattle farm. However, Shiaka *et al*. (2017) had *E. coli* from diarrhoeastool of a patience in Dutse, Jigawa state which was resistant to a higher combination of 8 sets of antibiotics probably due to the source among other reasons.

CONCLUSION/RECOMMENDATION

None of the *E. coli* was resistant to GNT and CIP while more than half (>50%) of the bacteria were resistant to AUG, TET, VAN and ERY. Most of the isolates (92%)were found to be multi-resistant to the antibiotics and could transfer to consumers. This could be of public health concern.

It is recommended that a number of multi-resistant bacteria in the abattoirs be reduced by consistently observing adequate hygiene and sanitation practices.

Further and frequent studies should be carried out to enhance standard operations in abattoirs which could contribute to improved public health.

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