Antibiograms of Commensal *Escherichia Coli* Isolated from Cattle in Some Selected Commercial Farms in Jos South Plateau State, Nigeria

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Abstract:

Antimicrobials are used for livestock production in the control and treatment of infections and as growth promoters but has been abusively used over the counter in middle and low income countries where access to these drugs are met with minimal restrictions and prescriptions. *Escherichia coli* (*E. coli*) is a normal inhabitant of the gastrointestinal tract of animals and a major reservoir of antibiotic resistant genes. The study aimed to identify antimicrobial resistance (AMR) phenotypes of commensal *E. coli* isolated from cattle in Jos South Local Government Area (LGA) of Plateau State Nigeria. Forty eight (48) fecal samples were collected from cattle in four randomly selected commercial farms, screened for *E. coli*, confirmed by conventional PCR and antimicrobial resistant profiles were determined using ten (10) commercial prepared antibiotic discs. Conventional PCR confirmed seven (7) isolates of *E. coli* equivalent to 14.58 % prevalence. The isolates showed varied phenotypic resistance to six antimicrobials, with a 100 % resistance to Reflacin (10 mm), Ceporex (10 mm) and Nalidix acid (10 mm). Thus, the findings of this study suggests the possibility of an extensive use of cephalosporin among farmers in the study area. The need for a larger implementation of surveillance programme in order to reduce and curb the spread of AMR determinant was recommended.

Keywords: Antimicrobial resistance, Cattle, *Escherichia coli*, polymerase chain reaction (PCR), Jos South.

Introduction

*Escherichia coli* (*E. coli*) are normal inhabitants of the gastrointestinal tract of animals. There are several strains of *E. coli* but a few of them cause serious foodborne infection in human; examples are the shiga toxin-producing *E. coli* (STEC) and verocytotoxigenic *E. coli* (VTEC) (Sobur et al., 2019). Cattle, asymptomatically carry STEC and represent the main natural host of VTEC. These pathogens are zoonotic and are easily transmitted to human from contaminated animal products and direct contact (Sobur et al., 2019). The global emergence of multidrug-resistant bacteria has been attributed to the overuse and misuse of antibiotics (Cantas et al., 2013). The use of antibiotics for veterinary purposes exceeds their use in humans, and these veterinary drugs are closely related to or belong to the same

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antimicrobial classes of those indicated for humans (Cantas et al., 2013). In Nigeria, the significant increase in the use of veterinary antimicrobials isn’t proportional to the increase in the annual livestock rate (Adesokan et al., 2015), and the nomadic system of cattle rearing are risk factors for the spread of antimicrobial resistant determinants between cattle and human (Igomu, 2020). Also, the practice of routinely administering antimicrobials to livestock as growth promoters and prophylaxis are important factor that promotes the emergence of antibiotic-resistant bacteria in the food chain with concomitant socioeconomic and public health risk (Fasure et al., 2012; Ojo et al., 2016).

Materials and Methods

Sample Collection

A total of forty eight (48) fecal samples of apparently healthy cattle were collected from four randomly selected commercial farms in Jos South LGA of Plateau State using stratified random sampling technique. The farms were designated as farm Vwang (12), Kuru (12), Gyet (12) and DU (12). All the farms were located in the four districts of the LGA. Faeces approximately 1 to 2 grams were aseptically collected from the rectum of each animal using clean disposable hand gloves into sterile stomacher bags. All samples were adequately labelled and placed in cold boxes and transported in to the Central Diagnostic Laboratory of National Veterinary Research Institute (NVRI) Vom laboratory for immediate analysis.

Samples Processing, Bacterial isolation and Identification

Two hundred millilitre (200 ml) of peptone water was prepared in schott duran bottles. 9 ml each of the prepared peptone water was dispensed into universal bottles and was autoclaved at 121°C for 15 minutes, after which it was allowed to cool. One (1) gram of faecal sample of the 48 samples was each weighed and dropped into universal bottle containing peptone water and was then incubated for 24 hours at 37°C. Following, the broth culture was then streaked directly on sterile MacConkey (MCA) agar plates (Oxoid Ltd., Detroit, Michigan, USA) and the inoculated plates were incubated at 37°C aerobically for 24 hours. A tentative affirmation was based on the appearance on the MCA culture plates, of a preponderance of colonies resembling E. coli (with a convex shape, dark pink color, entire edges and a diameter of 2 - 4 mm). From the 24 hours cultures, 2 to 3 representative colonies of E. coli were selected with a sterile inoculating loop and sub-cultured twice on eosin methylene blue (EMB) agar (Oxoid Ltd., Detroit, Michigan, USA) at 37°C, aerobically for 24 hours. Several colonies measuring 1-2 mm in diameter and with a characteristic metallic green sheen resembling that of E. coli were observed on the EMB plates. Further confirmation of E. coli was according to previous methods, and based on gram staining, and biochemical tests (catalase, oxidase, indole, methyl red, Voges-Proskauer test, citrate utilization, nitrate reduction, H2S production in TSI, gelatin liquefaction and urease test) (Cheesbrough, 2000; Nsofor et al., 2013; Beauvais et al., 2018). For PCR, DNA was extracted from pure culture using boiling methods following the procedures that was previously reported (Mahmud et al., 2018). PCR specific primers to detect E. Coli inv A gene as previously described was used; forward primer 5́GTG AAA TTA TCG CCA CGT TCG GGC AA 3́ and the reverse primer 5́TCA TCG CAC CGT CAA AGG AAC C 3́ (401 bp) (Yanestria et al., 2019).

Antimicrobial Susceptibility Test

Antibiotic susceptibility test of the isolates was performed following modified Kirby-bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI, 2008). Four to five well separated colonies of E. coli were selected from the EMB agar plates with a sterile inoculating loop, suspended in 2 mL of sterile normal saline in a tube, and vortexed to obtain a uniform mixture. The turbidity of the mixture was adjusted to 0.5 McFarland Standard by adding more organism if the suspension was found to be too light, or by further dilution with sterile saline if the suspension was too heavy. A sterile cotton swab was dipped into the
standardized suspension of *E. coli* and streaked evenly across the surface of Nutrient agar (Oxoid UK) to produce a uniform growth of the organism. With sterile forceps, 10 commercial antibiotic discs (Abtek pharmaceutical limited) were placed on the surface of the inoculated Nutrient agar and the plates were incubated at 37°C, aerobically for 18 hours. The antibiotics used in this study were; Reflacin (PEF) (10µg/disc), Tarivid (OFX) (10µg/disc), Ciproflox (CPX) (10µg/disc), Augmentin (AU) (30µg/disc), Gentamycin (CN) (20µg/disc), Ceporex (CEP) (30µg/disc), Nalidix acid (NA) (20µg/disc), Septrin (SXT) (30µg/disc), Amplicin (AP) (30µg/disc) and Streptomycin (S) (30µg/disc). Sensitivity pattern were determined by dividing isolates into three groups based on zone of inhibition produced by the antibiotic disc: susceptible, intermediate susceptible and resistant according to CLSI guideline (Olarinmoye et al., 2013).

Data Analysis

Data entry and analysis was performed with Microsoft Excel 2010. Descriptive statistics comprising percentages were used to indicate the proportion of cattle farms *E. coli* found in faecal sample. Summary data were presented as tables.

Results

Table 1. Diffusion Zone Breakpoints for Antimicrobial Sensitivity and Frequencies of Antibiotic Resistant *E. coli* Isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Drug code</th>
<th>Sensitive isolate</th>
<th>Resistant isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEF</td>
<td>0 (0.00)</td>
<td>7 (100)</td>
<td></td>
</tr>
<tr>
<td>OFX</td>
<td>5 (71.43)</td>
<td>2 (28.57)</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>7 (100)</td>
<td>0 (0.00)</td>
<td></td>
</tr>
<tr>
<td>AU</td>
<td>3 (42.86)</td>
<td>4 (57.14)</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>6 (85.71)</td>
<td>1 (14.29)</td>
<td></td>
</tr>
<tr>
<td>CEP</td>
<td>0 (0.00)</td>
<td>7 (100)</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>0 (0.00)</td>
<td>7 (100)</td>
<td></td>
</tr>
<tr>
<td>SXT</td>
<td>1 (14.29)</td>
<td>6 (85.71)</td>
<td></td>
</tr>
</tbody>
</table>
Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc: Susceptible (above 18mm), immediately susceptible I (11-17mm) and Resistant R (below 11mm).

In the present study, the prevalence of resistance phenotypes of *E. coli* ranged from nearly zero percent (0%) for Ciprofloxacin, 85.71% for Gentamicin to as high as 100% to Ceporex, Reflacin and Nalidixic acid. There was also noticeably high resistance to Ampilicilin and streptomycin both at 71.43%.

**Discussion**

The finding of this study is similar to those earlier reported by Nsofor et al. (2013) who identified resistant phenotypes of *E. coli* against third generation Cephalosporin and considerable high resistance to Nalidixic acid in cattle (Nsofor et al., 2013). This bacterium has been postulated to serve as a major reservoir of antimicrobial resistant genes (ARGs) within the gastrointestinal tract of cattle (Barour et al., 2019). Also, since *E. coli* has been shown to readily exchange genetic material with other bacterial species (Blake et al., 2003), it is possible that this organism may pass antibiotic resistance genes to transient bacterial pathogens that cause disease in humans (Barour et al., 2019). The findings of this study show *E. coli* isolate to be sensitive to Tarivid, Ciprofloxacan, and Gentamycin as similarly reported by farouk et al. (2018), while some were both sensitive and resistant to Augmentin, five resistant to both streptomycin and ampicillin with all the seven isolates resistant to Reflacin, Ceporex and Nalidix acid, which were not completely similar to the findings of farouk et al. (2018) who used similar antibiotics on clinical human isolates from stool in Kano state, Nigeria. Overall the findings of farouk et al. (2018) and this study emphasized the need for susceptibility testing, as resistant pattern can be to any combination of potentially effective antibiotics. Detection of *E. coli* in samples from cattle was not surprising as the pathogen is ubiquitous in nature and can be isolated as part of the normal intestinal microflora of cattle and the farm environment (Jajarmi et al., 2017).

The findings of this study also established the widespread presence of Cephalosporin resistant *E. coli* among cattle in farms in Jos South LGA. The attributes of *E. coli* make it a logical indicator of the extent of antibiotic resistance within microbial populations of the bovine digestive tract (Alexander et al., 2008), and since the intestinal tracts of cattle are important reservoirs for extended spectrum B-lactamases (ESBL) producing *E. coli*, and the presence of ESBL-producing bacteria in food has been attributed to the widespread use of antimicrobials in farming practices (Beauvais et al., 2018). Additionally, it was reported that the incidence of human infections caused by third generation cephalosporin-resistant *E. coli* is on the increase worldwide and recent studies have suggested that these ESBL-producing *E. coli* strains and their ARGs, can spread from food-producing animals through the food-chain to humans (de Been et al., 2014). Based on the outcome of this study, there is a possibility of wide and extensive usage of cephalosporin among farmers in Jos South LGA of Plateau State. Around the world there is an increase focus on AMR especially those attributed to the prevalence of ESBL producing bacteria among other factors (Alonso et al., 2017) with majority of this report been carried out on *E. coli* but in Nigeria very few reports concerning ESBL producing *E. coli* in cattle are been reported.

**Conclusion**

The prevalence of resistance phenotypes of *E. coli* in this study ranged from zero for Ciprofloxacin, 85.71% for Gentamicin to as high as 100% for Ceporex, Reflacin and Nalidixic acid. There was significantly high resistance to Ampilicilin and Streptomycin both at 71.43%.
Furthermore, it can be inferred that cattle in Jos South may harbour commensal *E. coli* capable of transmitting cephalosporin resistant determinants as well as serving as reservoirs to other antimicrobial drugs. The findings of this study should be interpreted with certain level of caution as few cattle were sampled however there is an urgent need for the regulation of antimicrobial drug usage in cattle production amongst livestock farmers and handlers in Jos South LGA of Plateau State. There is also the need for proper surveillance and monitoring of antibiotic resistance especially as it concerns ESBL producing *E. coli*.

Acknowledgement

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Conflict of Interests

No conflict of interest.

References


