

Antibiotic Resistance among Commensal *Escherichia coli* Isolated from Faeces of Cattle in Ado-Ekiti, Nigeria

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Abstract: The goal of this study was to isolate commensal *Escherichia coli* from faeces of apparently healthy cattle and determine their susceptibility to commonly used antibiotics. Non-repeat faecal samples were collected from 320 ready to be slaughtered cattle and 1,051 commensal isolates of *Escherichia coli* were recovered from 240 of the faecal samples collected using standard bacteriological methods. All the bacterial isolates were first examined for their susceptibility to antibiotics using protocols as specified by the Clinical Laboratory Standards Institute. The frequency of the antibiotic resistance among the isolates is as follows: ampicillin, 896 (85.3%); cotrimoxazole, 134 (12.8%); gentamicin, 926 (88.1%); nalidixic acid, 98 (9.32%); nitrofurantoin, 421 (40.1%); colistin, 662 (63.0%); streptomycin, 710 (67.6%) and tetracycline, 676 (64.3%). About 500 isolates were selected based on their antibiotic resistance phenotypes to determine their susceptibility to cephalosporins and flouroquinolones. The susceptibility of the isolates to cephalosporins are ceftazidime, 298 (59.6%); cefoxitin, 463 (92.6%); ceftriaxone, 107 (21.4%) and aztreonam, 241 (48.2%). The susceptibility to the flouroquinolones are: norfloxacin, 39 (7.8%); levofloxacin, 23 (4.6%); pefloxacin, 99 (4.6%); ofloxacin, 26 (5.2%) and ciprofloxacin, 55 (11.0%). The study has confirmed that *E. coli* recovered from cattle show high prevalence of antibiotic resistance.

Key words: Isolate, faecal, clinical laboratory, susceptibility, recovered, prevalence

INTRODUCTION

Escherichia coli naturally exists as a commensal in the gut of humans and animals where it enjoys synergetic association with other members of the bacterial family Entebacteriaceaea. It also plays a beneficial role in the prevention of potentially pathogenic organisms in the gut. Recent studies have also revealed that *E. coli* which forms part of the normal intestinal flora of humans and animals is capable of inhibiting the growth of other toxigenic strains of *E. coli* which is often associated with food-borne diseases in humans (American Society for Microbiology, 2009). However, some strains of *E. coli* occasionally emerge as pathogens due to the presence of certain pathogenic features and virulence genes which are located on transmissible genetic elements and this distinguish them from ordinary commensal strains (Ronsengren *et al.*, 2009).

The use of antibiotics mostly flouroquinolones and third-generation cephalosporins has assumed a common place in the sub-therapeutic treatment of animals. Such

antibiotics are frequently incorporated as animal food and drink supplements to cure and prevent diseases and also make them gain weight. The sub-therapeutic use of these antibiotics has generated a selective pressure which leads to emergence of antibiotic-resistant bacteria.

These can get to the food web and contaminate food and drinking water sources which can result in food and water borne diseases in humans. In most cases, infections with antibiotic-resistant bacteria often lead to antibiotic treatment failure in hospitals (Aarestrup *et al.*, 2000; Threfall *et al.*, 2000; Aubry-Damon *et al.*, 2004; Phillips *et al.*, 2004). Some clinical and environmental strains of flouroquinolones and cephalosporin-resistant *E. coli* are fully characterized. The major mechanisms that mediate resistance to common antibiotics in *E. coli* are the plasmids while some are chromosomally mediated. Resistance to cephalosporins is mostly coded by plasmids which code for enzymes that inactivate cephalosporins. Normally resistance to flouroquinolones is mediated by chromosomes but recent studies have confirmed that such resistance could also be carried by

resistance plasmids (Smith *et al.*, 2003). There is paucity of information on antibiotic resistance among bacterial isolates from food animals within the immediate study environment. The present study therefore investigated the antibiotic susceptibility of commensal *E. coli* isolated from faeces of cattle in Ado-Ekiti and environ to some commonly used antibiotics including cephalosporins and flouroquinolones.

MATERIALS AND METHODS

Collection and processing of faecal samples: During the period of this study (2006-2008), 320 fresh faecal samples were collected from apparently healthy cattle which were about to be slaughtered. All samples were collected at the Central Abattoir, Ado-Ekiti, Nigeria. All samples were taken using sterile swab sticks inserted into the rectal region of the cattle immediately the gut of each carcass was eviscerated. Each faecal sample was immediately inoculated onto freshly prepared eosine methylene blue (EMB, Oxoid, UK) agar plates and incubated at 35°C for 24 h. Presumptive identification was based on the appearance of characteristic green metallic sheen and all strains were further confirmed using standard biochemical techniques as previously described by Olutiola *et al.* (2000).

Antibiotic susceptibility testing: Antibiotic susceptibility tests were performed on all the bacterial strains using standard agar diffusion techniques as described by CLSI (2008). The susceptibility tests were performed in three phases. First, all strains were examined for their susceptibility to commonly used antibiotics using disks (Abtek, UK) containing the following: ampicillin (30 µg), cotrimoxazole (25 µg), gentamicin (25 µg), nalidixic acid (10 µg), nitrofurantoin (5 µg), colistin (10 µg), streptomycin (25 µg) and tetracycline (25 µg). The results were interpreted using standard interpretative charts as required by CLSI (2008).

In the second phase, 500 isolates that were confirmed to be multiple-antibiotic resistant were tested for their susceptibility to five flouroquinolones and four third generation cephalosporins. The cephalosporins were aztreonam (25 µg), cefoxitin (25 µg), ceftriaxone (25 µg) and ceftazidime (25 µg) while the flouroquinolones were norfloxacin (5 µg), levofloxacin (5 µg), pefloxacin (5 µg), ofloxacin (5 µg) and ciprofloxacin (5 µg) (Oxoid, UK).

RESULTS AND DISCUSSION

A total of 1051 *E. coli* isolates were recovered from faecal samples of 240 cattle. Based on the antibiotic susceptibility tests, the overall resistance of the isolates

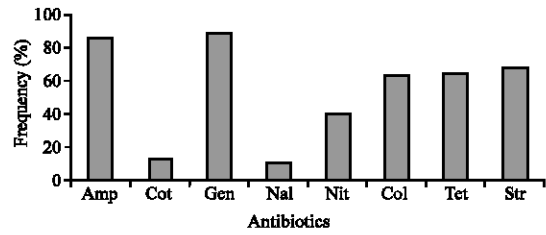


Fig. 1: Frequency of resistance to common antibiotics. Amp = Ampicillin, Cot = Cotrimoxazole, Gen = Gentamicin, Nal = Nalidixic acid, Nit = Nitrofurantoin, Col = Colistin, Tet = Tetracycline

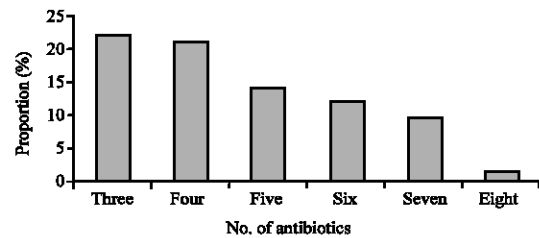


Fig. 2: Multiple antibiotic-resistance of *E. coli* to specific number of antibiotics (n = 1.051). Amp = Ampicillin, Cot = Cotrimoxazole, Gen = Gentamicin, Nal = Nalidixic Acid, Nit = Nitrofurantoin, Col = Colistin, Tet = Tetracycline

to antibiotics shows that resistance to gentamicin was highest with 892 (88.1%) isolates showing resistance to the antibiotic. While resistance to nalidixic-acid was least with 95 (9.0%) resistant to the antibiotic.

The isolates also show considerable resistance to ampicillin, tetracycline, cotrimoxazole, colistin and nitrofurantoin (Fig. 1). Many isolates also showed resistance to different combinations of antibiotics.

The number of isolates that showed resistance simultaneously to three antibiotics was highest among those isolates that demonstrated multiple antibiotic resistance while 20 isolates showed resistance to all eight common antibiotics (Fig. 2). About 71 different multiple antibiotic resistance phenotypes were also observed among those isolates that were multiply resistant (Fig. 3-8).

Antibiotic resistance has continued to constitute serious problems not only in human medicine but also in animal husbandry, livestock management and veterinary medicine (Witte, 1998).

It is also a global health challenge with greater effect on low-income countries. The ever-increasing incidence of antibiotic-resistant bacteria has been attributed to unregulated and inappropriate use of antibiotics. Huge quantities of antibiotics are reportedly consumed annually

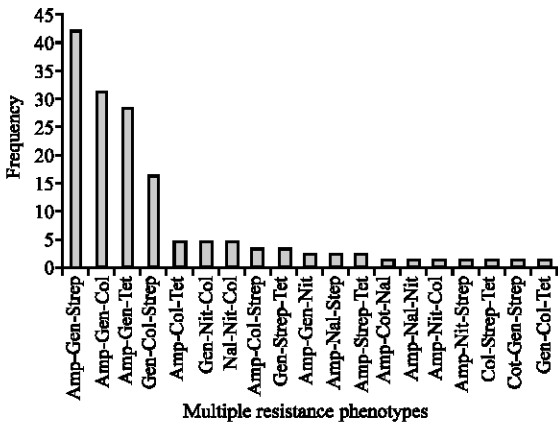


Fig. 3: Multiple antibiotics resistance against three antibiotics n = 1,051. Amp = Ampicillin, Cot = Cotrimoxazole, Gen = Gentamicin, Nal = Nalidixic Acid, Nit = Nitrofurantoin, Col = Colistin, Tet = Tetracycline

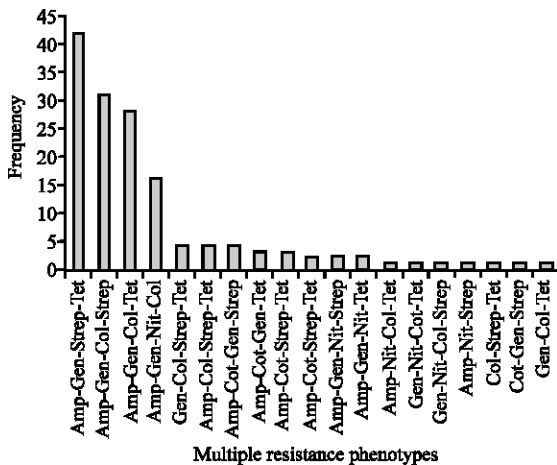


Fig. 4: Multiple antibiotics resistance against three antibiotics n = 1, 051. Amp = Ampicillin, Cot = Cotrimoxazole, Gen = Gentamicin, Nal = Nalidixic acid, Nit = Nitrofurantoin, Col = Colistin, Tet = Tetracycline

and this is principally, among other factors responsible for the ever increasing prevalence of antibiotic resistant bacteria (Lawson, 2008). The major finding in the present study reiterates the findings in other studies that have reported that antibiotic resistance among bacteria especially *E. coli* isolated from cattle and other animals is increasing at an alarming rate (Kozak *et al.*, 2009). A huge body of research have established that most commensal strains of *E. coli* isolated from cattle were resistant to antibiotics which is well correlated with the use of common antibiotics including tetracycline, streptomycin, fluoroquinolones and third-generation

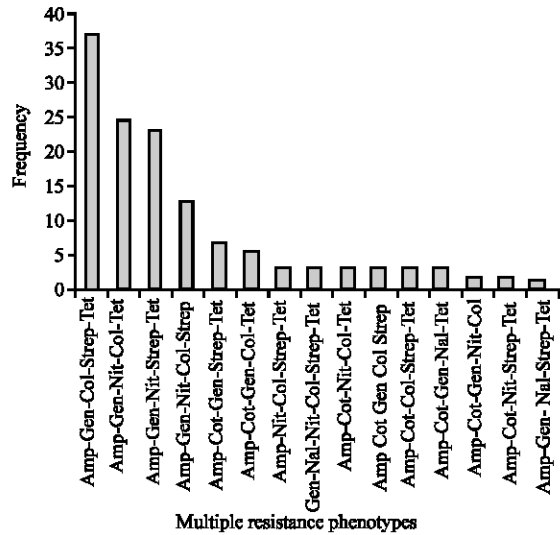


Fig. 5: Multiple antibiotics resistance against four antibiotics (n = 1.051). Amp = Ampicillin, Cot = Cotrimoxazole, Gen = Gentamicin, Nal = Nalidixic acid, Nit Nitrofurantoin, Col = Colistin, Tet = Tetracycline

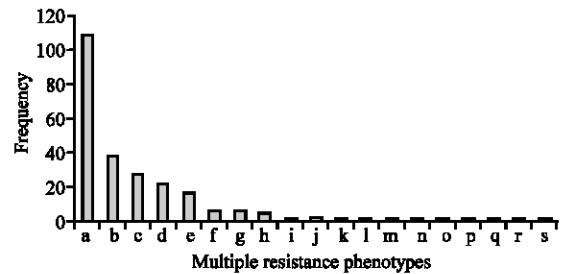


Fig. 6: Multiple antibiotics resistance against six antibiotics (n = 1,051)

cephalosporins (Schroeder *et al.*, 2002; Smith *et al.*, 2003). Sharma *et al.* (2008) observed that although, some commensal strains of *E. coli* may be intrinsically resistant to selected antibiotics, the exposure of *E. coli* to various combinations of antibiotics further increased the prevalence of resistant bacteria in cattle. Parveen *et al.* (2005) reported that *E. coli* isolated from the manure of cattle had high levels of resistance to commonly used antibiotics such as ampicillin, erythromycin and tetracycline. This could be attributed to the widespread use of antibiotics for growth enhancement and animal prophylactic therapy, all of which creates a selective pressure and favors the emergence of antibiotic resistance bacteria among the cattle sampled (Witte, 1998; Willis, 2000; Mora *et al.*, 2005).

The susceptibilities of all the isolates were tested using the using the agar diffusion technique. The results were interpreted using standard interpretative charts and

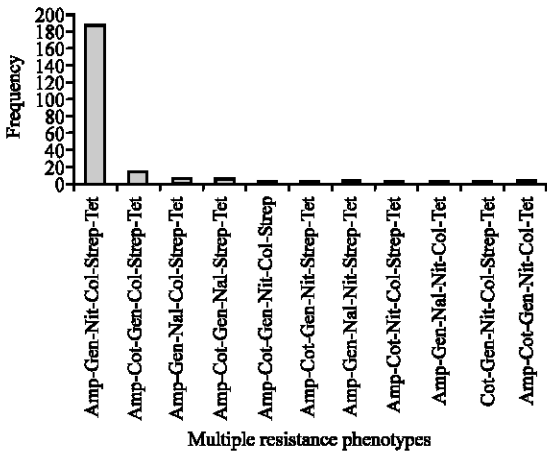


Fig. 7: Multiple antibiotics resistance against seven antibiotics (n = 1,051)

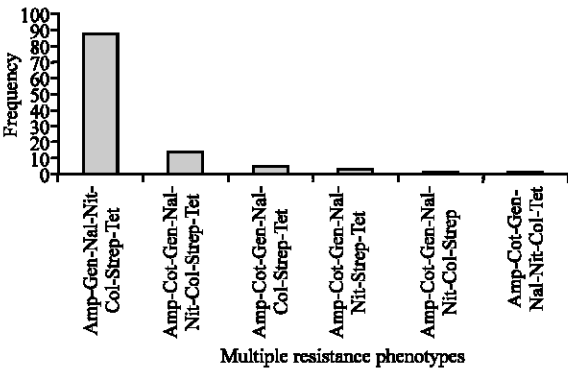


Fig. 8: Multiple antibiotics resistance against seven antibiotics (n = 1,051)

it was observed that the prevalence of multiple antibiotic resistance among the isolates was very high as 851 (81.0%) of all *E. coli* isolates showed resistance to at least three antibiotics. Another important finding is that 74 different multiple antibiotic resistance patterns or phenotypes involving the common antibiotics were observed among the isolates. However, this finding correlates with similar results obtained from other studies that have reported some levels of multiple antibiotic resistance in *E. coli* from cattle, meat products and other animals. For instance Umolu tested the susceptibility of *E. coli* isolated from meat and carcass to common antibiotics. The study revealed that isolates showed individual resistance to antibiotics in addition to nine different multiple antibiotic resistance patterns observed in the study. Scott screened faecal samples of cattle for multiple antibiotic resistant *E. coli* and observed that bacterial isolates developed resistance mostly to five antibiotics while some showed resistance to as many as nine antibiotics.

In another study, it was reported that commensal bacteria also develop multiple antibiotic resistance, despite not being directly linked to any disease but multiple resistance was defined as resistance to two antibiotics. Saenz reported seventeen different multiple antibiotic-resistant organisms in non-pathogenic *E. coli* recovered from humans, cattle and various food sources. In a study carried out in Nigeria, Aibinu determined the susceptibilities of *Escherichia coli* recovered from cattle, pigs and poultry in Lagos and Ogun states; they reported that some isolates were resistant to as many as 13 different antibiotics.

Hoyle *et al.* (2005) monitored the faecal flora of young cattle and observed that the gut was colonised by ampicillin-resistant strains of *E. coli* for a period of 4 months even though, the prevalence declined with age. In a similar study which examined the acquisition of ampicillin and nalidixic acid-resistant *E. coli* in the gut of cattle Hoyle *et al.* (2005) showed that *E. coli* acquired resistance to both antibiotics after exposure to specific concentrations of the two antibiotics. Sharma *et al.* (2008) recovered antibiotic-resistant *E. coli* and detected ampicillin and tetracycline resistant genes among the isolates. Isolates of *E. coli* recovered from pastoralist cattle tested for their susceptibility to ten antibiotics showed a high frequency of resistance to penicillin, erythromycin, cotrimoxazole and nitrofurantoin (Mubita *et al.*, 2008).

Even though, penicillin and erythromycin were not included in the array of eight common antibiotics used in this study, the ampicillin used in this present study is an antibiotic in the same class with penicillin while streptomycin shares the same class of with erythromycin (Brinas *et al.*, 2002; Meyer *et al.*, 2008; Khachatryan *et al.*, 2008). These antibiotics are related in their core structures and bacteria often show resistance to closely related antibiotics. According to Ngoma, *E. coli* recovered from apparently healthy cattle and subjected to antibiotic susceptibility tests revealed that the isolates were more frequently resistant to ampicillin, streptomycin and tetracycline; all of which were also among the common antibiotics tested in this present study to which the bacteria studied also showed resistance.

The number of isolates that showed resistance to tetracycline in this study was also high. About 64% of all isolates showed resistance to the antibiotic. Tetracycline and its derivatives is a drug of choice for use as feed supplement and in some cases into milk that is used as replacement diet for young animals. There are evidences that this practice has been associated with reduced susceptibility of *E. coli* and other bacteria in faeces. Furthermore, the results obtained in this study confirmed

that few isolates (98, 9.3%) showed resistance to nalidixic-acid indicating that resistance to the antibiotic was the lowest in prevalence among all the isolates studied.

This finding conforms by Hoyle *et al.* (2005) but added that prevalence of isolates that were co-resistant to ampicillin and tetracycline which were recovered from young cattle declined with age. Possible explanation for this phenomenon could be that antibiotics are often administered as growth promoter in young animals and such antibiotics are always gradually withdrawn as growth progresses.

The results of this study indicate that resistance to antibiotics particularly ampicillin, gentamicin, tetracycline, streptomycin and colistin are very high. The most obvious reason that could be responsible for the high frequency of antibiotic resistance especially to ampicillin, gentamicin and tetracycline is the heavy use of antibiotics for various purposes among in the cattle sampled. According to Enne the susceptibility of *E. coli* recovered from cattle, pigs and other animals revealed that 3.5% of 836 *Escherichia coli* isolates from cattle showed resistance to one or more antibiotics while 92% of 2,480 isolates from pigs were resistant to one or more antibiotics.

The major reason for these contrasting results was that pigs are generally more medicated due to their very poor natural hygiene; therefore more doses of antibiotics are required to prevent infections or illness among pigs than cattle. Similarly, the high rate of resistance among the bacteria obtained in this study could be attributed to a heavy antibiotic dosing for the cattle in the study area.

To further emphasise the role that antibiotics play in the emergence of resistant bacteria, Khachatryan *et al.* (2006) studied the effect of use of non-medicated dietary antibiotic supplement and reported that animals which received the antibiotic supplements had a higher incidence of tetracycline and streptomycin resistance among the *E. coli* recovered from faeces of the cattle in large farms. Similarly, in the study by Berge *et al.* (2006), faecal samples were recovered from cattle previously treated with tetracycline and neomycin and found that use of in-feed antibiotics were associated with increased level of antibiotic resistance among *E. coli*. Mubita *et al.* (2008) carried out a study in which *E. coli* isolated from pastoralist cattle were resistant to common antibiotics mostly amoxicillin, augmentin, tetracycline, streptomycin and also asserted that the use of these antibiotics could have led to the high prevalence and antibiotic resistance among the bacteria.

CONCLUSION

The overall result which showed a high prevalence of antibiotic resistance in this present study may be due to inappropriate use and abuse of antibiotics for the study animals with attendant serious implications. The sub therapeutic use of antibiotics for growth promotion and veterinary prophylaxis has probably contributed to an increase in the prevalence of antimicrobial-resistant bacteria in food animals and it may in turn, contribute to increased prevalence of antibiotic-resistant bacteria in humans. The antibiotic-resistant bacteria that emerge as a result of selection pressure in cattle can set up a food-chain in which antibiotic-resistant organisms that are constantly shed into the environment may get to the human community to cause serious and life-threatening antibiotic-resistant infections (Witte, 1998).

Another, grave implication is that the sub-therapeutic use of most antibiotics that are originally meant for the treatment and prevention of infections in humans, particularly the flouroquinolones and cephalosporins could compromise the use of these antibiotics and may lead to multiple antibiotic treatment failure. Therefore, decisive efforts must be made to ensure prudent and proper use of antibiotics and other antimicrobials. This also calls for continuous surveillance of bacterial resistance to antibiotics in the study area and the community (nation) at large owing to the significance to public health.

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