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Full Length Research Paper

Antimicrobial resistance among commensal *Escherichia coli* from cattle faeces and beef in Ibadan, Nigeria

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Commensal bacteria contribute to the distribution and persistence of antimicrobial resistance in the environment. This study monitored antimicrobial resistance in commensal *Escherichia coli* from the faeces of on-farm and slaughter cattle and beef. A total of 342 (89.5%) *E. coli* isolates were obtained from 382 samples. Isolation rate of *E. coli* was 90.0% in on-farm cattle, 87.1% in slaughter cattle and 92.2% in beef. Overall, the isolates showed resistance to amoxicillin (97.9%), ampicillin (97.9%), cefuroxime (25.1%), chloramphenicol (69.3%), ciprofloxacin (11.7%), cotrimazole (45.9%), erythromycin (59.4%), gentamycin (36.5%), nalidixic acid (27.2%), nitrofuratoin (54.9%), norfloxacin (21.1%), ofloxacin (14.0%), streptomycin (78.9%) and tetracycline (33.9%). There were no significant differences in antimicrobial resistance of *E. coli* from the different sample types. Only four (1.2%) of the 342 isolates were susceptible to all antimicrobial agents, while 338 (98.8%) were resistant to at least one of the tested antimicrobial agents. Multi-drug resistance to three or more antimicrobials was observed in 321 (93.9%) of all the isolates. Forty-one resistance groups were observed in on-farm cattle, 30 in slaughter cattle and 34 in beef. All the 30 resistance groups found in slaughter cattle were also present in on-farm cattle and beef. 'AmoAmpChlEryNitStr' and 'AmoAmpChlStr' were the predominant resistant patterns. This study confirmed on-farm and slaughter cattle as important sources of antimicrobial resistant *E. coli* transmissible to humans through beef.

Key words: Antimicrobial resistance, beef, *Escherichia coli*, on-farm cattle, slaughter cattle,

INTRODUCTION

Escherichia coli is an important commensal of gastro-intestinal tract of animals and humans where it contributes to the maintenance of gut physiology. Following acquisition of transferrable virulence properties encoded by plasmids, bacteriophages and transposons, commensal *E. coli* may become pathogenic and induce various intestinal and extra-intestinal diseases in infected host (Donnenberg and Whittam, 2001; Gamage et al., 2003; Branger et al., 2005). In debilitated and immuno-suppressed individuals or when gastrointestinal barriers are breached, commensal *E. coli* strains may cause secondary opportunistic infection (Ngwai et al., 2011).

Global increase in the incidence of antimicrobial resistance in bacterial strains is a major concern to all stakeholders in animal production, veterinary practice and public health (WHO, 2001). Realizing the dangers associated with the emergence and dissemination of resistant bacterial strains, many advanced countries have taken giant strides in formulating and promoting policies to limit the use of antimicrobial agents in food animals

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(WHO, 2001, 2002; Obeng et al., 2012). However, in developing nations of Africa, livestock farmers with little knowledge on the use of antimicrobial agents and the attendant consequences, have unrestricted access to these agents and use them without recourse to expert advice or supervision.

Commensal *E. coli* is often used as indicator bacteria for monitoring and assessing the level of antimicrobial resistance in the community (Kijima-Tanaka et al., 2003). The ease of transferability of resistance trait among bacteria through mobile genetic elements and the preponderance of *E. coli* in the faeces and environment may facilitate the development and spread of antimicrobial resistance (Kang et al., 2005; Lee et al., 2006).

Antimicrobial resistant bacteria can enter the meat production chain from carrier animals and get transmitted to humans (Hammerum and Heuer, 2009). Overdependence on antimicrobial usage in animal production as well as unhygienic practices during meat production and marketing are important factors responsible for the emergence of antimicrobial resistance in bacteria of animal origin and their eventual transmission to humans (WHO, 2000).

This study investigates antimicrobial susceptibility of commensal *E. coli* from the faeces of on-farm and slaughter cattle at abattoir as well as from beef sold to the general public in markets in Ibadan, Nigeria. This is to provide baseline information on the association between antimicrobial resistant *E. coli* originating from animals on farm and those in meat meant for human consumption. It is hoped that this will help in policy formulation towards controlled use of antimicrobial agents in animal production for the protection of public health.

MATERIALS AND METHODS

Sampling

On-farm cattle

A total of 160 faecal samples were collected from all eight cattle herds identified in a farm settlement in the peri-urban area of Ibadan, South western Nigeria. Both dairy and beef cattle were present on all the herds. The herdsmen stated that they use antimicrobial agents but kept no record of medication. The number of animals in each herd was estimated to be 120 including adult and young cattle of both sexes. Faecal samples were collected directly from the rectum of animals with sterile swabs. Twenty healthy animals were sampled in each herd by systematic random sampling. Only one sample was collected per animal.

Slaughter cattle

A total of 132 faecal samples were collected from the rectum of slaughter cattle at the Bodija Municipal abattoir, Ibadan, Nigeria. Over 300 heads of cattle were slaughtered daily in the abattoir (Osibanjo and Adie, 2007). Slaughtering of cattle was done without separation between clean and dirty operations, thereby facilitating easy contamination of meat (Adeyemo, 2002). Two visits were made to the abattoir weekly for four weeks. On each visit, a minimum of 15 animals (representing an approximated 5% of all animals slaughtered in the abattoir) were sampled using systematic random sampling technique. Only one sample was collected per animal using sterile swab.

Beef

Ninety fresh beef samples were randomly purchased from meat vendors in three open markets in Ibadan, Nigeria. All the vendors from whom meat were obtained said that they received their beef supply from the same abattoir investigated in this study. Vendors were asked to put 10 g of meat into a universal bottle held open for them. Three visits were made to each of the markets and ten samples were collected on each occasion. Only one sample was obtained from an individual vendor per visit.

Sample preservation

Samples were properly labeled and preserved in ice-pack. They were transported to the laboratory for immediate microbiological analysis.

Isolation and identification of E. coli

Each faecal sample (1 g) was inoculated unto nine milliliters of sterile tryptic soy broth (TSB) (Oxoid, Basingstoke, UK) in universal bottles. 10 g of each beef sample was thoroughly homogenized and inoculated into 90 ml of TSB in a conical flask. The broth cultures were incubated at 37°C for 18 to 24 h. After incubation, a loopful of the TSB culture was inoculated onto MacConkey agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 18 to 24 h. Rose pink colonies on MacConkey agar (putative *E. coli*) were selected for biochemical test. After an initial screening for oxidase and catalase production, biochemical test kit for identification of gram negative bacteria (Microbact GNB 12E, Oxoid®) was used for the identification of selected colonies. Results of biochemical tests were interpreted with computer software (Oxoid Microbact® 2000 version 2.03) for the confirmation of *E. coli*.

Antimicrobial susceptibility test

The susceptibility of identified *E. coli* isolates to antimicrobial agents was determined by the standard Kirby-Bauer disk diffusion method. A single colony of the isolate under test was inoculated unto TSB and incubated for 8 to 12 h. After incubation, the turbidity of the TSB culture was adjusted to 0.5 McFarland standard. A sterile swab was dipped into the adjusted TSB culture and inoculated onto Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) plate by swabbing the entire surface of the MHA. The antimicrobial disks were individually placed firm on the inoculated MHA plate. The plates were incubated at 37°C for 18 to 24 h. After incubation, the diameter of the clear zone of inhibition around each antimicrobial disk was measured (in millimeters) and the result was interpreted in accordance with the recommendation of Clinical and Laboratory Standards Institute (CLSI), (2008). Susceptibility to the following antimicrobials was determined: amoxicillin (25 µg), ampicillin (10 µg), cefuroxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cotrimazole (25 µg), erythromycin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofuratoin (300 µg), norfloxacin (5 µg), ofloxacin (5 µg), streptomycin (10 µg) and tetracycline (30 µg). *E. coli* American Type Culture Collection (ATCC) 25922 was included for quality control.

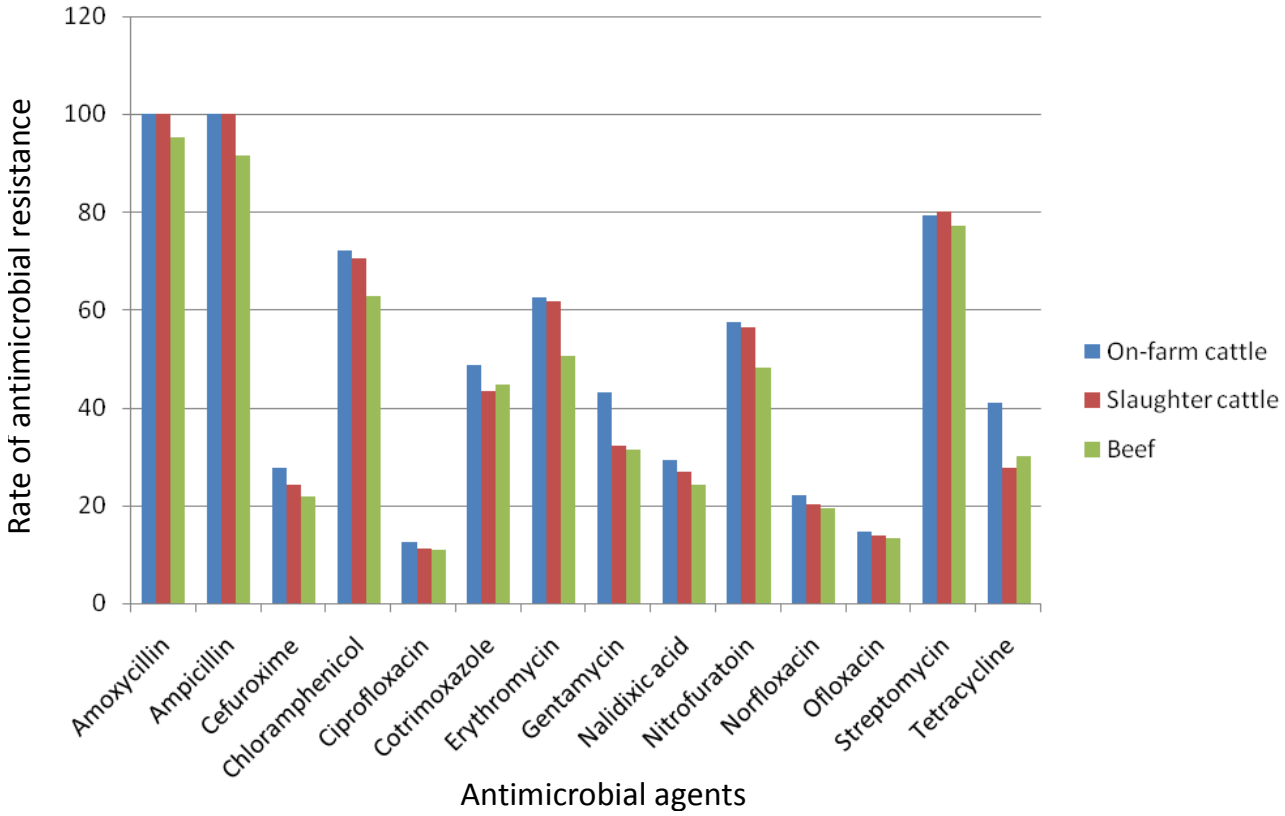

Figure 1. Rates of antimicrobial resistance in *E. coli* isolates from the faeces of cattle and beef in Ibadan, Nigeria.

Statistical analysis

Rates of antimicrobial resistance at the different stages of sampling were compared by Chi-square test at $p<0.05$ probability level using Statistical Software Package for Social Sciences (SPSS, version 16, 2007).

RESULTS

Escherichia coli isolates

A total of 342 (89.5%) *E. coli* isolates were obtained from 382 samples. The isolates included 144 (90.0%) of 160 faecal samples of on-farm cattle, 115 (87.1%) of 132 faecal samples of slaughter cattle and 83 (92.2%) of 90 fresh beef samples.

Antimicrobial resistance rates

The overall rate of *E. coli* resistance to the antimicrobial agents was as follows: amoxicillin (97.9%), ampicillin (97.9%), cefuroxime (25.1%), chloramphenicol (69.3%), ciprofloxacin (11.7%), cotrimazole (45.9%), erythromycin (59.4%), gentamycin (36.5%), nalidixic acid (27.2%), nitrofuratoin (54.9%), norfloxacin (21.1%), ofloxacin (14.0%), streptomycin (78.9%) and tetracycline (33.9%).

The rate of antimicrobial resistance of *E. coli* isolates from on-farm cattle was amoxicillin (100.0%), ampicillin (100.0%), cefuroxime (27.8%), chloramphenicol (72.2%), ciprofloxacin (12.5%), cotrimazole (48.6%), erythromycin (62.5%), gentamycin (43.1%), nalidixic acid (29.2%), nitrofuratoin (57.6%), norfloxacin (22.2%), ofloxacin (14.6%), streptomycin (79.2%) and tetracycline (40.9%) (Figure 1).

In slaughter cattle, the rate of *E. coli* resistance was amoxicillin (100.0%), ampicillin (100.0%), cefuroxime (24.3%), chloramphenicol (70.4%), ciprofloxacin (11.3%), cotrimazole (43.5%), erythromycin (61.7%), gentamycin (32.2%), nalidixic acid (26.9%), nitrofuratoin (56.5%), norfloxacin (20.1%), ofloxacin (13.9%), streptomycin (80.0%) and tetracycline (27.8%) (Figure 1).

The rate of antimicrobial resistance of *E. coli* isolates from beef was amoxicillin (95.2%), ampicillin (91.6%), cefuroxime (21.7%), chloramphenicol (62.7%), ciprofloxacin (10.8%), cotrimazole (44.6%), erythromycin (50.6%), gentamycin (31.3%), nalidixic acid (24.1%), nitrofuratoin (48.2%), norfloxacin (19.3%), ofloxacin (13.3%), streptomycin (77.1%) and tetracycline (30.1%) (Figure 1).

There were no significant differences ($p>0.05$) in the rates of resistance to antimicrobial agents among *E. coli* isolates from on-farm cattle, abattoir slaughter cattle and beef (Figure 1).

Antimicrobial resistance groups

Overall, 338 (98.8%) of all 342 isolates were resistant to at least one of the antimicrobial agents tested. Only four isolates were susceptible to all antimicrobial agents and these were from beef. Three hundred and twenty one (93.9%) of all the isolates were resistant to three or more antimicrobial agents and can be regarded as being multi-drug resistant. A total of 45 *E. coli* resistance groups were observed in the present study (Table 1). Forty one resistance groups were observed in on-farm cattle, 30 in slaughter cattle and 34 in beef. All the 30 resistance groups found in slaughter cattle were common to all the sample categories.

DISCUSSION

The use of antimicrobial agents especially in animal production has been identified as an important factor which select for antimicrobial resistant bacterial strains (WHO, 1998). Globally, antimicrobial resistant bacteria resident in the gut of carrier animals contribute signify-cantly to environmental contamination and spread of anti-microbial resistant bacterial strains (Kang et al., 2005; Lee et al., 2006). This has necessitated continuous monitoring and surveillance of antimicrobial resistance in zoonotic and commensal bacteria of animal origin for the protection of public health (WHO, 2001).

In the present study, the rates of antimicrobial resis-tance in *E. coli* were similar at the three stages of sampling. There was high resistance of over 70% to amoxicillin, ampicillin and streptomycin among *E. coli* isolates. Likewise, the isolates showed moderate to high resistance (between 30 and 70%) to gentamycin, cotrimazole, nitrofuratoin, erythromycin, chloramphenicol and tetracycline. These drugs are widely used in animal production in Nigeria and are readily available over the counter.

This study also showed a relatively low resistance (below 30%) to the quinolones (nalidixic acid, ciprofloxacin, norfloxacin and ofloxacin) and cefuroxime. This further proves that there is a steady increase in resistance to quinolones which were originally shown to have excellent activities against *E. coli* isolates of human and animal origins (Orden et al., 2000, 2001). It has been reported that there is an increase in the use of the fluoroquinolones in livestock production consequent to resistance and chemotherapeutic failure accompanying treatment with first-line older generation antibiotics (Alo and Ojo, 2007).

In an earlier study, *E. coli* isolates from faeces of animal, human septage and surface water showed resistance to nalidixic acid (0.67%), gentamycin (0.77%), nitrofuratoin (0.87%), chloramphenicol (1.06%), ampicillin (5.5%), streptomycin (13.21%) and tetracycline (28.06%) (Sayah et al., 2005). These are lower than the resistance rates observed for the respective antimicrobial agents in the present study. However, Umolu et al. (2006) reported higher resistance rates to ampicillin (100%), chloramphenicol (100%), nitrofuratoin (90%), tetracycline (78%), cefuroxime (73%), nalidixic acid (35%) and norfloxacin (33%) in *E. coli* isolates from beef than observed in the present study. The 43% *E. coli* resistance to cotrimazole (Umolu et al., 2006), 71.4% to streptomycin (Roopnarine et al., 2009) and 8.8% to ciprofloxacin (Kuyucuoglu et al., 2012) previously reported are similar to the findings in the present study. Differences in the level of dependence on antimicrobial usage and management practices in animal production as well as variations in legislation guiding the use of antimicrobials from region to region may influence the antimicrobial selection pressure exerted on enteric bacteria such as *E. coli* and hence the discrepancies in the rate of *E. coli* resistance from different geo-cultural areas.

Majority (98.8%) of the *E. coli* isolates in the present investigation was resistant to at least one antimicrobial agent tested. Furthermore, 321 (93.9%) of all the 342 isolates examined demonstrated resistance to three or more antimicrobial agents (multidrug resistance). Forty five resistant groups were found in the present study. A greater diversity in the antimicrobial resistance pattern was observed in *E. coli* from the faeces of on-farm cattle and beef than in slaughter animals. All the thirty resistance groups observed in slaughter cattle were also present in on-farm cattle and beef. In addition to these thirty resistance groups found in all sample categories, eleven resistance groups were found only in on-farm animals, while four resistance groups were found only in beef. The greater diversity in resistance patterns and presence of unique resistance groups in on-farm animals may be due to higher number of isolates and the inclusion of young animals in the sample population at the herds' level. At slaughter, only adult animals were available for sampling. Increasing age of animals has been associated with a progressive decline in antimicrobial resistance in *E. coli* isolated from cattle (Watson et al., 2012). The presence of resistance groups unique to beef can be explained in terms of likely contamination from the environment and humans during transportation, handling and open display of meat in the market (Bender, 1992; Abdullahi et al., 2006).

This study revealed that on-farm and slaughter cattle are carriers of multidrug resistant *E. coli* and also suggested that beef is a vehicle for possible transmission to humans. Proper legislation is required to regulate access to antimicrobial agents and restrict their use in animal production in order to prevent the increasing incidence of resistance. Strict adherence to the principles of hygiene in abattoirs operations and during post-process handling of beef will reduce the potential health risk associated with faecal and environmental contamination.

Table 1. Antimicrobial resistance patterns of *E. coli* isolates from the faeces of cattle and beef in Ibadan, Nigeria.

Resistance groups	Resistance patterns	Source of isolate			Total
		On-farm cattle	Slaughter cattle	Beef	
R1	AmoAmpCefChlCipCotEryGenNalNitNorOflStrTet	8	5	2	15
R2	AmoAmpCefChlCotEryGenNalNitNorOflStrTet	2	2	1	5
R3	AmoAmpCefChlCipCotEryGenNalNitOflStrTet	2	2	2	6
R4	AmoAmpChlCipCotEryGenNalNitNorOflStrTet	4	2	2	8
R5	AmoAmpCefChlCotEryGenNalNitNorStrTet	2	-	-	2
R6	AmoAmpChlCipCotEryNalNitNorOflStrTet	1	1	1	3
R7	AmoAmpChlCipCotEryGenNalNitNorOflStr	1	1	1	3
R8	AmoAmpCefChlCotEryGenNalNitStrTet	3	-	-	3
R9	AmoAmpChlCotEryGenNalNorOflStrTet	2	2	1	5
R10	AmoAmpCefCotEryGenNalNitNorStrTet	4	4	2	10
R11	AmoAmpChlCotEryGenNalNitNorStrTet	1	-	-	1
R12	AmoAmpCefChlCotEryGenNitStrTet	7	4	1	12
R13	AmoAmpChlCotEryGenNalNitStrTet	3	3	3	9
R14	AmoAmpCefChlCotEryGenNalNorStr	4	4	2	10
R15	AmoAmpChlCotEryGenNalNitStrTet	2	2	1	5
R16	AmoAmpCefChlCotEryGenNitTet	1	-	-	1
R17	AmoAmpChlCotEryGenNitStrTet	3	3	3	9
R18	AmoAmpCefCipCotEryNalStr	2	2	1	5
R19	AmoAmpChlCotEryGenNitTet	5	2	3	10
R20	AmoAmpChlCotEryGenStrTet	1	-	-	1
R21	AmoAmpChlEryNitStrTet	1	1	2	4
R22	AmoAmpCefChlEryNitStr	3	3	2	8
R23	AmoAmpChlCotGenTet	1	-	-	1
R24	AmoAmpChlEryNitStr	17	17	3	37
R25	AmoAmpChlGenStrTet	2	-	-	2
R26	AmoAmpChlNitNorStr	-	-	2	2
R27	AmoAmpCotEryGenStr	1	1	2	4
R28	AmoAmpCotEryStrTet	1	1	2	4
R29	AmoAmpCefEryStr	2	2	2	6
R30	AmoAmpChlEryNit	1	1	1	3
R31	AmoAmpChlEryNit	5	5	1	11
R32	AmoAmpChlGenTet	1	-	-	1
R33	AmoAmpChlNitStr	-	-	1	1
R34	AmoAmpCotNitStr	9	9	7	25
R35	AmoAmpEryOflStr	1	1	1	3
R36	AmoAmpGenStrTet	1	-	-	1
R37	AmoAmpNalNorStr	1	1	1	3
R38	AmoAmpChlStr	14	14	8	36
R39	AmoAmpGenTet	1	-	-	1
R40	AmoAmpStrTet	2	-	-	2
R41	AmoAmpChl	7	7	8	22
R42	AmoAmpStr	9	7	5	21
R43	AmoAmp	6	6	2	14
R44	Amo	-	-	3	3
R45	Susceptible to all	-	-	4	4
Total		144	115	83	342

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