Veterinaria Italiana, 2012, **48** (3), 297-308

Antibiotic-resistant commensal *Escherichia coli* in faecal droplets from bats and poultry in Nigeria

Anthonia Olufunke Oluduro

Summary

The prevalence of antibiotic resistance and plasmid carriage among commensal faecal *Escherichia coli* isolates of bats, broilers and free-range chickens in Ile-Ife, Osun State, Nigeria was studied. A total of 125 *E. coli* isolates were recovered from the fresh faecal samples of bats, broilers and free-range chickens on eosin methylene blue agar plates and characterised using standard biochemical tests. The susceptibility of the isolates to antibiotics was performed using the disk diffusion method. All isolates developed resistance to antibiotics to varying degrees; resistance to augumentin, amoxicillin and tetracycline was significantly higher ($p<0.05$), compared to the other antibiotics. The lowest resistance was recorded with ofloxacin, ciprofloxacin and pefloxacin in bats and free-range isolates. In general, resistance was higher ($p<0.05$) in broilers than in free-range isolates, but was comparable in bat isolates ($p>0.05$) with the exception of ciprofloxacin, pefloxacin gentamicin and ofloxacin. A total of 90% of the bat isolates developed multiple antibiotic resistance with 28 multiple antibiotic resistance patterns. The free-range chicken and broiler isolates displayed 10 and 38 multiple antibiotic resistance patterns, respectively. Resistance was mostly plasmid-mediated with molecular weights ranging between 0.91 kb and 40.42 kb. Antibiotic resistance and plasmid carriage among the commensal *E. coli* isolates studied was relatively high and may be implicated in zoonotic infections.

Keywords

Antibiotic, Bat, Broiler, Chicken, *Escherichia coli*, Faeces, Nigeria, Plasmid, Resistance.

Escherichia coli commensale antibiotico-resistente in goccioline fecali di pipistrelli e pollame in Nigeria

Riassunto

Nel presente lavoro è stata studiata la prevalenza dell' antibiotico-resistenza e il trasporto del plasmide tra Escherichia coli *commensale fecale isolata in pipistrelli, polli da carne e polli allevati all'aperto a Ile-Ife, Stato Osun, Nigeria. Un totale di 125 isolati di* E. coli *è stati isolato da campioni fecali di pipistrelli, pollame da ingrasso e polli free-range su piastre agar eosina blu di metilene e caratterizzato utilizzando test biochimici standard. La suscettibilità degli isolati di antibiotici è stata eseguita utilizzando il test di sensibilità anti-microbica (disk diffusion method). Tutti gli isolati hanno sviluppato resistenza a vari gradi; la resistenza a augumentin, amoxicillina e tetraciclina è risultata significativamente maggiore (p<0,05) rispetto ad altri antibiotici. Nei pipistrelli e nei polli free-range è stata registrata una resistenza inferiore nei confronti di ofloxacina, ciprofloxacina e pefloxacina. In generale, la resistenza era più elevata (p<0,05) nei polli da carne rispetto ai free-range, ma era paragonabile agli isolati di pipistrelli (p>0,05) ad eccezione di ciprofloxacina, genta-micina e pefloxacina ofloxacina. Il 90% degli isolati nei pipistrelli ha sviluppato una resistenza multipla agli antibiotici con un pattern di 28 antibiotico-resistenza, mentre gli isolati da pollame free-range e*

Department of Microbiology, Faculty of Science, Obafemi Awolowo University, 220005, Ile-Ife, Nigeria
aoluduro2003@yahoo.co.uk

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polli da carne hanno mostrato rispettivamente 10 e 38 pattern di antibiotico-resistenza. La resistenza era per lo più mediata da plasmidi, con peso molecolare compreso tra 0,91 kb e 40,42 kb. L'antibiotico-resistenza e il trasporto di plasmide E. coli *commensale tra gli isolati analizzati sono risultati relativamente elevati e, come tali, possono essere implicati nelle infezioni zoonosiche.*

Parole chiave
Antibiotico, *Escherichia coli*, Nigeria, Pipistrello, Plasmide, Pollame, Pollo, Resistenza.

Introduction

Antibiotic resistance has serious clinical and public health implications as it imposes limits on the quantity and quality of antibiotics which are widely used for the control, treatment and prevention of infection within human and animal populations. It has been suggested that the use of antimicrobial agents for clinical use and in animal food may have contributed to the development and spread of resistant bacteria in animals and the environment (40).

Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations (15, 30). Resistance to antimicrobial agents among *Escherichia coli* isolates from wildlife has been suggested to be acquired from the foods they consume and from the environment; resistance may also reflect the use of antimicrobials in humans, livestock and pets (21). Resistance to antimicrobial agents among *E. coli* strains from bats and other wildlife has been reported (10, 33). In Trinidad and Tobago, *E. coli* strains have been isolated from wildlife, including bats, and some have exhibited resistance to antimicrobial agents (1, 2, 13). Bats and other wild animals have been implicated in the epidemiological cycles of several emerging and re-emerging zoonoses (24). Bats have also been documented as carriers of pathogenic agents, such as vampire-borne rabies (18), severe acute respiratory syndrome (SARS) virus, or SARS-like coronaviruses (22), *Histoplasma capsulatum* (4) and leptospirosis (34), making them important in the epidemiology of bacterial, viral and mycotic zoonoses.

Food-borne transmission of multi-resistant sorbitol-fermented *E. coli* is an important source of infection in humans (27, 38). Domestic animals have been implicated as the principal reservoir (29, 38). Much of the evidence relating to the potential for transfer of a resistance problem from animals to humans comes from a consideration of the epidemiology of zoonoses, particularly commensal multiple antibiotic resistant *E. coli*. *E. coli* are facultative anaerobic Gram-negative bacteria found in the intestinal tract of humans and animals (36). Commensal *E. coli* is often used as an indicator organism to assess the extent and type of resistance in the gastrointestinal tract, since it plays a dynamic role in the ecology of multi-drug-resistant bacteria and has been shown to be a reservoir of resistance (23, 32).

Several antibiotics are used for the treatment of clinical infections and many are currently used sub-therapeutically in animals, particularly in livestock and poultry. Therefore, the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria (39, 40). At slaughter, resistant strains from the gut readily soil poultry carcasses and, as a result, poultry meats are often contaminated with multi-resistant *E. coli* (37). Likewise, eggs become contaminated during laying (19, 40). Hence, resistant faecal *E. coli* from poultry can infect humans both directly and via food. These resistant bacteria may colonise and induce conferment of resistance genes on human endogenous flora (8). Considering the fact that bacteria are capable of acquiring resistance by acquisition of new chromosomal or extra-chromosomal DNA from resistant bacteria of the same or even other species, and so much so that food animals, as well as food of animal origin, is traded worldwide, the occurrence of antimicrobial resistance in one country can be considered a problem for all countries (25). During the last decade, awareness of the potential problems that could emerge on the human health front from antimicrobial-

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resistant bacteria among food-producing animals has increased. Hence many countries have established monitoring programmes to determine the occurrence of resistant bacteria in food animals (25).

There are strong indications that the incidence and prevalence of multiply antibiotic resistance is increasing, not only among pathogenic *E. coli*, but also among commensal strains of *E. coli* from animals. Hence, it is necessary to ascertain the degree of multiply antibiotic resistance among commensal strains of *E. coli* in bats and apparently healthy poultry (broilers, free-range chickens) being reared for consumption. The present study therefore presents the antibiotic resistance profile of commensal *E. coli* isolates from bats, broilers and free-range chicken faeces in Ile-Ife, Nigeria.

Material and methods

Collection of faecal samples

Fresh faecal samples of bats and apparently healthy chickens (broilers, free-range) were collected in sterile universal bottles containing swab sticks. Faecal samples of randomly selected chickens were collected from the slaughterhouses of the Obafemi Awolowo University agricultural farm, in Ile-Ife, Nigeria, and other poultry farms in the Ile-Ife metropolis. Bat faecal samples were collected from bats nesting on trees behind the Biological Science buildings at the Obafemi Awolowo University, Ile-Ife. Hand-held mesh nets also were used to capture bats in dwelling houses. The faecal samples were taken to the laboratory immediately for bacteriological analysis.

Isolation and identification of *Escherichia coli*

Faecal samples were streaked on the solid surface of eosin methylene blue agar (Oxoid Ltd, Basingstoke, Hampshire) plates and incubated aerobically at 37°C for 24 h. Plates were examined for the characteristic appearance of *E. coli* (metallic green sheen) and colonies with green metallic sheen were interpreted as *E. coli* isolates. The isolates were further confirmed using standard methods (6, 28).

Antibiotic susceptibility

Antibiotic susceptibility of isolates was determined using the antibiotic disc diffusion technique on Mueller Hinton agar (Himedia Laboratories Pvt Ltd, Mumbai) (7). The antibiotics (Oxoid Ltd), augmentin (AUG) (30 µg), ciprofloxacin (CPX) (10 µg), nitrofurantoin (NIT) (200 µg), ofloxacin (OFL) (5 µg), tetracycline (TET) (30 µg), gentamicin (GEN) (10 µg), ceftriazone (CRO) (30 µg), amoxicillin (AMX) (25 µg), cotrimoxazole (COT) (25 µg), pefloxacin (PFX) (5 µg) were firmly placed on the agar plates previously seeded with the test organisms and incubated at 37°C for 24 h. Sensitivity of the isolates to different antibiotics was indicated by the clear zones of inhibition which were measured in millilitres using a calibrated ruler. The diameters of zone of inhibition were compared with the interpretative chart of zone sizes of susceptibility to antibiotics (7).

Plasmid analysis

Plasmid DNA extraction was performed on randomly selected multiple antibiotic resistant *E. coli* isolates from bats, broilers and free-range chickens using the alkaline method described by Birnboim and Doly (3). Plasmid DNA were separated on the basis of molecular weight using 1% agarose gel electrophoresis and visualised under ultraviolet light using an ultraviolet trans-illuminator.

Plasmid curing

Curing of plasmid was performed using acridine orange which is a mutagen. *E. coli* isolates that harboured plasmid DNA were subcultured in the acridine orange broth mixture in bijou bottles and incubated at 37°C for 4 days. After 4 days of curing, the subcultured isolates were cultured on nutrient agar plates and incubated at 37°C for 24 h to obtain cured *E. coli* isolates. Susceptibility of the cured isolates to the previously used antibiotics was performed as described earlier using the disk diffusion method.

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Statistical analysis

The Chi-square test was used to assess significant difference in the prevalence and degree of resistance among the isolates. The analysis was performed using the Statistical Package for Social Sciences software (SPSS, Version 12).

Results

A total of 125 *E. coli* isolates, comprising 25 from free-range chickens and 50 each from bats and broilers, were tested for their susceptibility to antibiotics. All of the isolates developed resistance to the antibiotics in varying proportions (Table I). Resistance to augumentin, amoxicillin and tetracycline was significantly higher ($p<0.05$), compared to the other antibiotics. Resistance was low to ofloxacin, ciprofloxacin and pefloxacin among bats and free-range isolates. Generally, resistance to the antibiotics was higher ($p<0.05$) in broilers than in free-range isolates, but comparable in bat isolates ($p>0.05$) with the exception of ciprofloxacin, pefloxacin, gentamicin and ofloxacin (Table I). The bat isolates showed highest resistance to augumentin (92%) and least to ciprofloxacin, ofloxacin and pefloxacin (1%). Similarly, broiler isolates showed highest resistance to augumentin (74%), followed by tetracycline (72%) and least to ofloxacin (14%), while among free-range chicken isolates, the highest resistance was to amoxicillin (80%) and least to ofloxacin (12%).

Multiple antibiotic resistance ranged from resistance to two antibiotics to nine antibiotics (Table II). A high percentage of the isolates exhibited multiple antibiotic resistance with 36% of bat isolates showing multiple antibiotic resistance to three antibiotics, 30% of broiler isolates to four antibiotics and 32% of the free-range chicken isolates to two antibiotics.

A total of 28 multiple antibiotic resistance patterns were observed among the 50 bat isolates studied. Resistance to three antibiotic groups (40%) was the most common multiple resistance pattern recorded among the isolates, while resistance to six antibiotic groups (6.6%) was the least prevalent. The multiple antibiotic resistance pattern AMX, AUG and NIT was the most pattern observed most frequently (Table III).

Thirty-eight multiple antibiotic resistance patterns were observed among the broiler isolates of which resistance to four antibiotic groups (31.9%) was the most common multiple antibiotic resistance pattern recorded with the pattern AUG, GEN, AMX and TET being the most prevalent (Table IV).

Similarly, 56% of the free-range chicken isolates which were multiple antibiotic resistant, displayed ten multiple antibiotic resistance patterns, with resistance to two antibiotic groups being the most common (Table V).

The molecular weight of the plasmid DNA of the selected multiple antibiotic resistant

Table I
Prevalence of antibiotic resistant faecal *Escherichia coli* isolates

Antibiotic	No. of isolates (%) Bats (n = 50)	Broilers (n = 50)	Free-range chickens (n = 25)	Zone diameter breakpoint (mm)
Augumentin (30 µg)	46 (92)	37 (74)	15 (60)	≤13
Ceftriazone (30 µg)	15 (30)	12 (24)	8 (32)	≤13
Nitrofurantoin (200 µg)	27 (54)	22 (44)	17 (68)	≤14
Gentamicin (10 µg)	11 (22)	27 (54)	10 (40)	≤12
Cotrimoxazole (25 µg)	20 (40)	22 (44)	6 (24)	≤10
Ofloxacin (5 µg)	1 (2)	7 (14)	3 (12)	≤12
Amoxicillin (25 µg)	41 (82)	32 (64)	20 (80)	≤13
Ciprofloxacin (10 µg)	1 (2)	16 (32)	3 (12)	≤15
Tetracycline (30 µg)	15 (30)	36 (72)	14 (56)	≤14
Pefloxacin (5 µg)	1 (2)	15 (30)	0 (0)	≤12

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Table II
Prevalence of multiple antibiotic resistant *Escherichia coli* isolates

Source	No. of antibiotics	No. of isolates (%)
Bats (*n* = 50)	2	8 (16)
	3	18 (36)
	4	12 (24)
	5	4 (8)
	6	3 (6)
Broilers *n* = 50)	2	2 (4)
	3	6 (12)
	4	15 (30)
	5	7 (14)
	6	5 (10)
	7	4 (8)
	8	4 (8)
	9	4 (8)
Free-range chickens (*n* = 25)	2	8 (32)
	3	2 (8)
	4	4 (16)

The molecular weight of the plasmid DNA of the selected multiple antibiotic resistant isolates from bats, broilers and free-range chickens ranged between 0.91 kb and 40.42 kb (Table VI). The gel electrophoresis picture revealed the various plasmid DNA bands (Figs 1 and 2).

A number of the isolates showed multiple plasmid DNA as depicted by lanes 8, 30 and 32 in Figure 1. However, some of the isolates, particularly the broiler isolates (BR 36, BR 3 and BR 24) and free-range chicken isolates (FR 1, FR 6, FR 15 and FR 34) as well as the bat isolate (B 36), were resistant to certain antibiotics before and after plasmid curing (Table VI).

Discussion

Generally, resistance to antibiotics was significantly higher in broiler isolates than in bat and free-range chicken isolates. The study corroborates the findings of van den Bogaard *et al*. (40) who reported a similar resistance rate in faecal *E. coli* isolates in a poultry population with amoxicillin being the highest resistance antibiotic among the broiler isolates. The high prevalence and degree of resistance observed in broiler isolates in the present study and other studies may be due to antibiotic use, crowding and poor sanitation, as these factors are typical of intensive poultry farming (5, 40).

Resistance to antibiotics (ciprofloxacin, ofloxacin and pefloxacin) which was significantly higher in broiler isolates than in free-range chicken and bat isolates (Table I) may be due to the therapeutic use of these antibiotics in poultry. In many cases, antibiotic compounds are added to poultry feed in subtherapeutic doses for prophylaxis, contributing to a significantly higher population of resistant bacteria in these animal species, by exerting a potent selective pressure for emerging resistant clones that already pre-existed in the bacterial population (20). The prevalence of fluoroquinolone-resistant *E. coli* in humans and animals has been increasing worldwide (9).

Antibiotics, particularly flouroquinolone use in food animals, has been of particular concern because they are critically important in the treatment of serious infections in humans (41), and their use in food animals has resulted in a substantial increase in the number of fluoroquinolone-resistant bacteria in these livestock and in food derived from them (16).

Table III
Multiple antibiotic resistance patterns among *Escherichia coli* isolates from bats

No. of antibiotics	Multiple antibiotic resistance patterns	No. of isolates
2	AMX, AUG	3
	AUG, NIT	3
	COT, AUG	1
	TET, AUG	1
3	AMX, AUG, NIT	6
	TET, AUG, NIT	1
	COT, AUG, NIT	4
	GEN, AUG, NIT	1
	COT, TET, AUG	1
	COT, AMX, AUG	2
	TET, AUG, CRO	2
	GEN, AMX, AUG	1
4	COT, AMX, AUG, CRO	2
	COT, AUG, AMX, TET	2
	GEN, AMX, AUG, NIT	1
	COT, GEN, AMX, AUG	1
	AMX, TET, AUG, NIT	1
	COT, GEN, AUG, NIT	1
	AMX, AUG, CRO, NIT	1
	GEN, TET, AUG, NIT	1
	GEN, AMX, TET, AUG	1
	COT, AMX, CRO, NIT	1
5	AMX, TET, AUG, CRO, NIT	1
	COT, GEN, AMX, AUG, NIT	1
	COT, GEN, AMX, TET, AUG,	1
	COT, AMX, AUG, CRO, NIT	1
6	COT, AMX, TET, AUG, CRO, NIT	1
	GEN, AMX, TET, AUG, CRO, NIT	2

AUG augmentin (30µg)
CPX ciprofloxacin (10 µg)
NIT nitrofurantoin (200 µg)
OFL ofloxacin (5 µg)
TET tetracycline (30 µg)
GEN gentamicin (10 µg)
CRO ceftriazone (30 µg)
AMX amoxicillin (25 µg)
COT cotrimoxazole (25 µg)
PFX pefloxacin (5 µg)

Studies by Johnson *et al*. (16) and Lautenbach *et al.* (20) indicate that fluoroquinolone use in food animals is an important source of emerging fluoroquinolone-resistance among *E. coli* and should be considered a foodborne pathogen.

The high multi-antibiotic resistance observed in *E. coli* from bats, broilers and free-range chickens can also be explained by the external use of antibiotics in poultry and cross-resistance in bat isolates. Although free-range chickens hardly receive any modern veterinary attention, they may maintain close contact through a myriad of routes with organisms originating from other important hosts in their environment, such as humans via excreta and exotic chickens that had been previously exposed to various antibiotics. Some

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Table IV
Multiple antibiotic resistance patterns among *Escherichia coli* isolates from broilers

No. of antibiotics	Multiple antibiotic resistance patterns	No. of isolates
2	AUG, NIT	1
	AMX, TET	1
3	AUG, AMX, TET	1
	COT, AMX, TET	2
	GEN, COT, OFL	1
	GEN, AMX, TET	1
	CRO, AMX, PFX	1
4	AUG, GEN, AMX, TET	4
	AUG, COT, AMX, TET	3
	AUG, CRO, NIT, TET	1
	AUG, NIT, AMX, TET	2
	AUG, GEN, TET, PFX	1
	AUG, GEN, AMX, CPX	1
	AUG, NIT, AMX, CPX	1
	AUG, GEN, COT, AMX	1
	NIT, GEN, AMX, TET	1
5	AUG, NIT, COT, AMX, TET	2
	AUG, NIT, GEN, AMX, TET	2
	CRO, GEN, OFL, CPX, TET	1
	AUG, NIT, OFL, AMX, TET	1
	CRO, NIT, OFL, AMX, CPX	1
6	AUG, CRO, COT, OFL, TET, PFX	1
	AUG, NIT, GEN, CPX, TET, PFX	1
	AUG, CRO, GEN, OFL, CPX, PFX	1
	AUG, NIT, COT, AMX, TET, PFX	1
	GEN, COT, OFL, AMX, TET, PFX	1
7	AUG, CRO, NIT, GEN, COT, AMX, TET	1
	AUG, CRO, NIT, OFL, CPX, TET, PFX	1
	AUG, GEN, COT, OFL, AMX, CPX, TET	1
	AUG, GEN, COT, OFL, CPX, TET, PFX	1
8	AUG, CRO, NIT, GEN, COT, OFL, CPX, PFX	1
	AUG, GEN, COT, OFL, AMX, XPX, TET, PFX	1
	AUG, NIT, GEN, COT, AMX, CPX, TET, PFX	1
	AUG, NIT, GEN, COT, OFL, AMX, CPX, TET	1
9	AUG, CRO, NIT, GEN, COT, OFL, AMX, CPX, PFX	1
	AUG, NIT, GEN, COT, OFL, AMX, CPX, TET, PFX,	1
	AUG, CRO, GEN, COT, OFL, AMX, CPX, TET, PFX	1
	AUG, CRO, NIT, GEN, COT, OFL, AMX, CPX, TET	1

AUG augmentin (30 µg)
CPX ciprofloxacin (10 µg)
NIT nitrofurantoin (200 µg)
OFL ofloxacin (5 µg)
TET tetracycline (30 µg)
GEN gentamicin (10 µg)
CRO ceftriazone (30 µg)
AMX amoxicillin (25 µg)
COT cotrimoxazole (25 µg)
PFX pefloxacin (5 µg)

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Table V
Multiple antibiotic resistance patterns among *Escherichia coli* isolates from free-range chickens

No. of antibiotics	Multiple antibiotic resistance patterns	No. of isolates
2	NIT, GEN	1
	AUG, GEN	1
	AUG, OFL	1
	NIT, OFL	1
	AUG, CRO	2
	AUG, NIT	1
	OFL, TET	1
3	AUG, NIT, OFL	1
	COT, AMX, CPX	1
4	AUG, GEN, AMX, TET	4

AUG augmentin (30µg)
CPX ciprofloxacin (10 µg)
NIT nitrofurantoin (200 µg)
OFL ofloxacin (5 µg)
TET tetracycline (30 µg)
GEN gentamicin (10 µg)
CRO ceftriazone (30 µg)
AMX amoxicillin (25 µg)
COT cotrimoxazole (25 µg)
PFX pefloxacin (5 µg)

Table VI
Plasmid profiles of multiple antibiotic resistant *Escherichia coli* isolates

Lanes	Isolate code	Molecular weight of plasmid bands (approx.) (kb)	Antibiotic resistance before plasmid curing	Antibiotic resistance after plasmid curing
M	–	–	–	–
8	BR 6	1.54, 0.91	AUG, CRO, NIT, TET	AUG, NIT
22	BR 25	15.68	AUG, GEN, TET, PFX	AUG, GEN, TET
26	BR 36	3.71	AUG, CRO, NIT,GEN, COT, OFL, AMX, CPX, TET	Sensitive
30	BR 11	16.82, 8.93	AUG, COT, AMX, TET	TET, AUG
32	FR 6	6.29, 5.09	OFL, TET	Sensitive
34	FR 1	27.18	AUG, GEN, AMX, TET	Sensitive
38	B 36	24.27	GEN, AMX, TET, AUG, CRO, NIT	Sensitive
39	FR 15	20.47	AUG, GEN, AMX, TET	AUG, TET
40	FR 34	20.47	AUG, GEN, AMX, TET	Sensitive
42	FR 15	21.66	AUG, NIT, OFL	Sensitive
43	BR 3	22.29	AUG, AMX, TET	Sensitive
45	BR 24	40.42	AUG, NIT, GEN, CPX, TET, PFX	Sensitive

M marker, λ Hind III digest 1

AUG augmentin (30µg)
CPX ciprofloxacin (10 µg)
NIT nitrofurantoin (200 µg)
OFL ofloxacin (5 µg)
TET tetracycline (30 µg)
GEN gentamicin (10 µg)
CRO ceftriazone (30 µg)
AMX amoxicillin (25 µg)
COT cotrimoxazole (25 µg)
PFX pefloxacin (5 µg)

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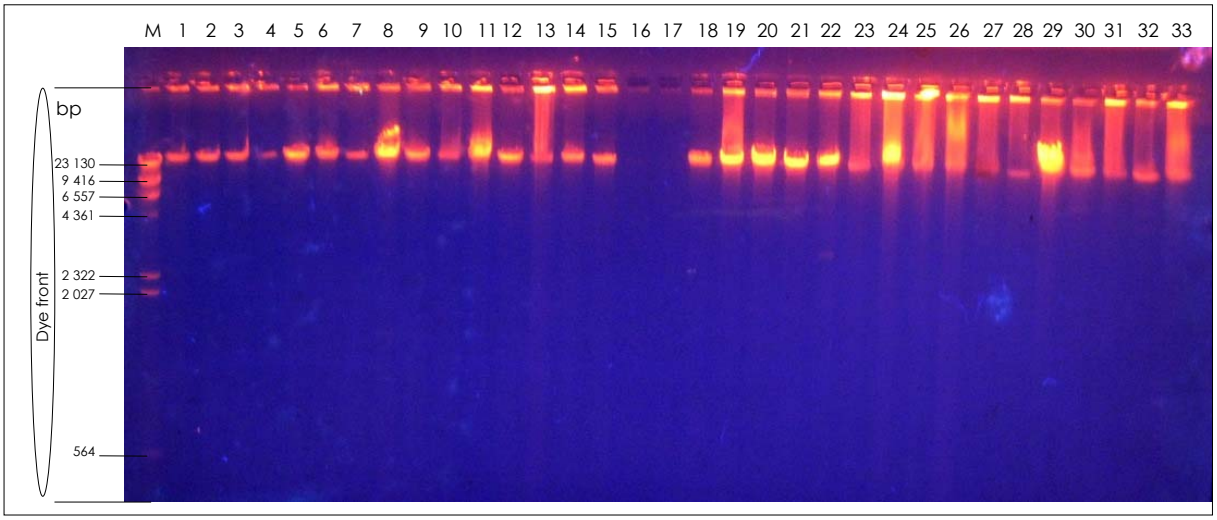


Figure 1
Plasmid profiles of multiple antibiotic resistant *Escherichia coli* isolates harbouring plasmids
Lane M: marker, λ Hind III digest 1
Lane 8: BR 6, 1.54 kb and 0.91 kb
Lane 22: BR 25, 15.68 kb
Lane 26: FR 36, 3.71 kb
Lane 30: BR 11, 16.82 kb and 8.93 kb
Lane 32: FR 6, 6.29 kb and 5.09 kb

antimicrobial-resistant *E. coli* isolates from human faeces may originate from poultry (17).

The high prevalence of resistance to antibiotics, particularly augumentin, amoxicillin and nitrofurantoin, among *E. coli* strains from bats in the present study is comparable to results from other studies conducted in other countries (10, 33). It has also been reported that resistance to antimicrobial agents among wildlife species may vary locally and may be linked to the use of antibiotics in humans and animals (31, 33). Similarly, in our study, resistance was frequently developed to augmentin, tetracycline and amoxicillin among poultry isolates. Tabatabaei *et al.* (35) reported a similar resistance trend to tetracycline, ampicillin, amoxicillin and cloxacillin in non-pathogenic *E. coli* isolates from chicken flocks. This widespread resistance to these antibiotics can be attributed to the extensive and long-term use of the antibiotic for veterinary therapy, prophylaxis and as a growth promoter in many animal species, resulting in the selection of resistant pathogenic and commensal bacteria (39).

The multiple antibiotic resistance observed in the *E. coli* isolates in the study is either plasmid-borne or chromosomally mediated, as some isolates that were originally resistant to some of the antibiotics tested, later became susceptible after plasmid curing. Bacteria which carry plasmid-mediated resistance genes provide a potential reservoir of genes which can be transferred not only within species but between different bacterial species and genera (11) and this has significance for the spread of antimicrobial resistance genes to many bacteria, including human pathogens. Tabatabaei *et al.* (35) reported the isolation of transmissible plasmids from multi-drug resistant non-pathogenic *E. coli* isolates in chicken flocks.

Bacteria carrying plasmids for multiple resistance may be auto transferred by conjugation, both *in vitro* and *in vivo*, and this promotes the spread of resistance genes within microbial populations. In the long term, bacteria resistant to antibiotics become established in the microflora of animals and human beings, even when no antibiotic is being administered (14). Of more concern to both human and veterinary medicine is the observation that the prohibition of an antimicrobial agent does not necessarily lead to a dramatic reduction in the prevalence of resistance to that drug. This calls into question

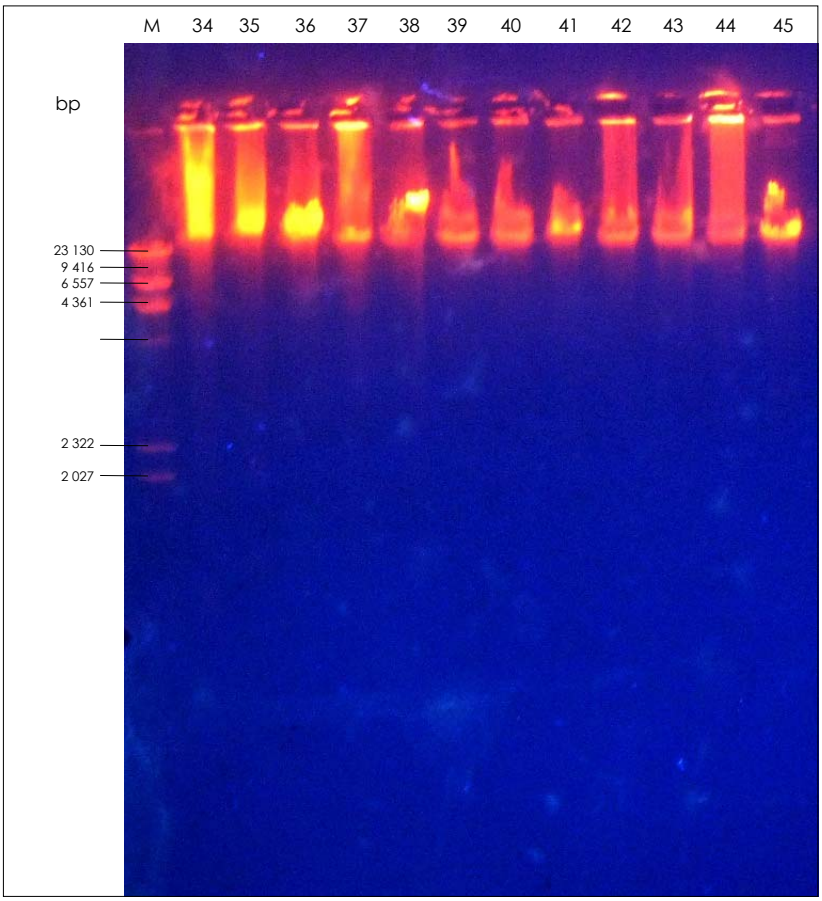


Figure 2
Plasmid profiles of multiple antibiotic resistant *Escherichia coli* isolates harbouring plasmids
Lane M: marker, λ Hind III digest 1
Lane 34: FR 1, 27.18 kb
Lane 38: B 36, 24.27 kb
Lane 39: BR 15, 20.47 kb
Lane 40: FR 34, 20.47 kb
Lane 42: FR 15, 21.66 kb
Lane 43: BR 3, 22.29 kb
Lane: 45: BR 24, 40.42 kb

the assumption that resistance persists only as a result of the selective pressure applied by continued use of the antimicrobial agent. It is possible that there are other influences that could ensure the survival of antimicrobial resistance genes, either by design or by fortuity. However, the ease with which bacteria acquire new resistance genes by self-transmissible and mobilisable plasmids and conjugative transposons may represent a more significant contribution to the increasing incidence of resistant strains (26).

Since exposure to antimicrobial agents is the most important factor with regard to development of antimicrobial resistance, animals and animal products could thus be significant sources of resistant bacteria for the human population (12), with attendant multiple antibiotic treatment failure in infection therapy.

Conclusion

This study has established and confirmed that the prevalence of antibiotic resistance among *E. coli* isolates from poultry and bats is relatively high and the incidence of carriage of plasmids is correspondingly high in the study area. The plasmid basis of resistance can promote the spread of antibiotic resistance. Considering, therefore, the cost implication of antibiotic resistance in infection therapy and

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its overall health significance in humans, stringent monitoring of the prophylactic use of antibiotics in animals and in feeds by the government, health and animal care profession is strongly advocated.

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