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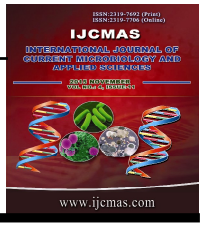

Original Research Article

Antibiotic Resistance Pattern of Two Indicator Bacteria Isolated from Cow Dung across Ten Local Government Areas of Ekiti State, Nigeria

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Keywords

Cow dungs, Antibiotics susceptibility, *Enterococcus faecalis*, *Escherichia coli*, Animal reservoir, Food chain.

A B S T R A C T

The prevalence of acquired resistance of two indicator bacteria isolated from cow dungs across ten (10) local government areas of Ekiti State was assessed using standard microbiological methods. Selected strains of *Enterococcus faecalis* (100) and *Escherichia coli* (100) isolated from cow dungs were characterized by standard cultural and biochemical tests and they were tested for their sensitivity to different antibiotics using disk diffusion *method*. Antibiogram profiles show a high resistance of *E. faecalis* to cloxacillin (75%), tetracycline (67%), cotrimoxazole (61%), amoxycillin (55%) and augmentin (51%). Low resistance to vancomycin (5%) and gentamicin (13%) were noticed among *E. faecalis* isolates. Antibiogram profile also shows a high resistance of tested *E. coli* strains to amoxycillin (93%), followed by cotrimoxazole (89%), nitrofurantoin (87%), tetracycline (84%), augmentin (83%) and nalidixic acid (75%). Eighty percent (80%) and seventy-nine (79%) percent of the tested *E. coli* strains were susceptible to gentamicin and ofloxacin respectively. Multi-resistance occurred in more than 10% of isolated strains of *E. faecalis* and in more than 50% of tested *E. coli* strains. The results of this study underscore the prevalence of an animal reservoir of antibiotic resistant microorganism that have the potential to enter the food chain.

Introduction

Our anthropocentric view of human pathogens has historically caused us to think of bacterial resistance to antimicrobials as a problem arising purely out of clinically related events. In fact, it is being increasingly recognized that antimicrobial resistance develops at a high frequency among bacteria in the food animal production environment. The conundrum is whether the prevalence of resistance in this environment contributes to the problem being observed in the clinical setting. *Enterococcus faecalis* and *Escherichia coli* have presented serious challenges clinically,

as both are leading cause of nosocomial infections, gastrointestinal infections, urinary tract infections and are becoming increasingly resistant to treatment with antimicrobials (Ruiz-Garbajosa *et al.,* 2009; Sharma *et al.,* 2009). Over 24% of nosocomial infections are complicated by the intrinsic resistance of this group of organisms to many antibiotics as well as acquired resistance to tetracycline, amoxicillin, **c**otrimoxazole (Hendrickx *et al.,* 2008; Marrow *et al.,* 2009; Sharada *et al.,* 2009). While *E. faecalis* is predominantly associated with endocarditis as well as bladder and epididymal infections, *E. coli* are common cause of sepsis and neonatal meningitis (Rahman *et al.,* 2008).

Acquired resistance of *Enterococcus faecalis* to vancomycin (a last line antibiotics for most serious gram positive infection) is an alarming problem indicating that fatal bacterial infection may not be well treated as experienced in days before the emergence of the drug. As well *Escherichia coli* may also pass onto other organism the genes responsible for antibiotics resistance. Since the prevalence of resistance against antibiotics in *E. faecalis* and *E. coli* isolated from cow dungs is relatively unknown, we compared the resistance situation of both organisms. The data obtained are discussed in respect to the major trends in antibiotic resistance pattern of the organisms. Resistance prevalence of *E. faecalis* is compared to recent data on resistance prevalence of *E. coli* especially in the food industry.

Materials and Methods

A total of 100 strains of *Enterococcus faecalis* and 100 strains of *Escherichia coli* were isolated across ten (10) local government areas of Ekiti State in Nigeria. They originated from the cow dungs.

Sample collection

Samples were collected from different locations across 10 local governments and samples collected for investigations and research work were fresh cow dungs. The samples were collected into a sterile sample bottle and then corked.

Isolation and identification

Samples collected were streaked on already prepared eosin methylene blue agar and Slanetz and Bartley Agar plates. The plates were incubated at 37°C for 24 hours except for Slanetz and Bartley which required about 48 hours for distinct growth. Isolates were presumptively characterized as *Enteroccus faealis* based on Gram stain, catalase reaction, tolerance to 6.5% NaCl and growth at 45°C, the production of pyrrolidonylarylamidase, and hydrolysis of esculin in the presence of bile while isolates were presumptively classified as *Escherichia coli* based on Gram stain, indole production, methyl red and sugar fermentation.

Antibiotics susceptibility test

The isolates were grown at 37°C in Mueller-Hilton broth (Oxoid) for 16–18h and diluted to an optical density of 0.1 (0.5 McFarland Standard) at a wavelength of 625nm and stored at 4oC. The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute (2008). The isolates were tested against eight commercial antibiotic disks (Abtek Biologicals Limited Gram positive and gram negative discs) with their concentrations (in μg) Abtek. Gram positive disc contains: augmentin (30μg), amoxycillin (30μg), erythromycin (5μg), gentamicin (10μg), tetracycline (10μg), cloxacillin (5μg), chloramphenicol (30μg) and cotrimoxazole (25μg). The gram negative disc contains: ofloxacin (30μg), amoxycillin (25μg), augmentin (30μg),

cotrimoxazole (25μg), nalidixic acid (30μg), gentamicin (10μg), nitrofurantion (30μg) and tetracycline (30μg). Multidrug resistance is defined as isolates resistance to more than three classes of drugs.

Data analysis

Data analysis was carried using SPSS version 15.0[16], with values expressed in means and percentage. Statistical significance difference was determined by chi-square test ($p<0.05$).

Results and Discussion

A total of 100 isolates of *Enteroccus faecalis* and *Escherichia coli* were selected and subjected to various morphological and biochemical tests.

Table 1 shows the antibiotic resistance pattern of *E. faecalis* isolated from cow dungs across 10 local government areas of Ekiti State, Nigeria. It was observed that most of the *Enterococcus faecalis* isolates were sensitive to few of the antibiotics. The highest level of resistance was observed in cloxacillin (75%), tetracycline (67%), cotrimoxazole (61%), amoxicillin (55%), and augmentin (51%). Table 2 shows the antibiotic resistance pattern of *E. coli* isolated from cow dungs across 10 local government areas of Ekiti State, Nigeria High level resistance was against amoxycillin (93%), cotrimoxazole (89%), nirofurantoin (87%), tetracycline (84%), augmentin (83%) and nalidixic acid (75%). All the isolates from ADO, EKITI WEST, EKITI SWEST, IKOLE and IDO/OSI local governments exhibited complete resistance (100%) to amoycillin, cotrimoxazole and nitrofurantoin. Only few of the isolates showed resistance to ofloxacin and gentamicin. Only one (1) isolate from ADO local government showed resistance to ofloxacin and gentamicin respectively. In this study a total of 100 isolates of *Enterococcus faecalis* and *Escherichia coli* from 10 local government areas of Ekiti state were studied. In previous studies, antimicrobial resistance of *Enterococcus faecalis* and *Escherichia coli* from cattle have been examined, but in separate reports (Devriese *et al.,* 1992; Hershberger *et al.,* 2005; Tenhagen *et al.,* 2006). In addition, many of those studies have reported on enterococci from bovine mastitis or milk samples and not enterococci from cattle faecal samples. The data gathered from this study reflects the antimicrobial resistance patterns of both organisms isolated from faecal samples. This information will be helpful in future risk assessments of antimicrobial use practices and public health.

It was observed that most of the *E. faecalis* isolates were sensitive to few of the antibiotics. The highest level of resistance were observed in cloxacillin (75%), followed by tetracycline (67%), cotrimoxazole (61%), amoxicillin (55%) and augmentin (51%). This finding was in agreement with the work of Papaioannou *et al.* (2007) who reported 81.5% resistance of *E. faecalis* to tetracycline. A minimal level of resistance was observed against gentamicin (13%). This finding was similar to the work of Jackson *et al.* (2010) who reported 0% resistance of *E. faecalis* to gentamicin. The study demonstrate alarming multiple resistance to antibiotics in *Escherichia coli* than that of *Enterococcus faecalis* as high level of resistance was against amoxicillin (93%), cotrimoxazole (89%), nitrofurantion (87%), tetracycline (84%), augmentin (83%) and nalidixic acid (75%). This finding was in agreement with the work of Sharada *et al.* (2009) who reported a high resistance value of *E.coli* to nitrofurantoin (90.7%), tetracycline (83.08) and cotrimoxazole (76.92%).

Table.1 Antibiotics resistance pattern of *E. faecalis* isolated from cow dungs across 10 local government areas of Ekiti State, Nigeria

LG	Antibiotics								
	AUG	AMX	ERY	TET	CXC	GEN	COT	CHL	VAN
(n=10)									
ADO									
S	5	5	4	2	1	7	1	4	10
I	1	1	2	1	0	3	0	1	0
R	4	4	4	7	9	0	9	5	0
EFON’									
S	6	6	7	1	4	4	6	6	10
I	0	1	2	1	0	2	1	0	0
R	4	3	1	8	6	4	3	4	0
EKITI WEST									
S	4	3	2	1	1	6	3	5	8
I	0	0	2	2	1	1	1	0	1
R	6	7	6	7	8	3	6	5	1
EKITI SWEST									
S	3	2	3	2	0	8	2	7	10
I	2	2	1	6	0	1	1	2	0
R	5	6	6	6	10	1	7	1	0
IKERE									
S	1	1	1	0	2	9	4	5	8
I	0	0	0	0	0	1	0	1	1
R	9	9	9	10	8	0	6	4	1
IKOLE									
S	2	1	3	2	8	7	2	6	9
I	1	0	0	0	0	3	0	2	0
R	7	9	7	8	2	0	8	2	1
IJERO									
S	5	6	5	2	0	7	2	7	9
I	1	0	2	1	0	2	0	1	0
R	4	4	3	6	10	1	8	2	1
IRE/IFELODUN									
S	2	2	3	2	1	5	2	7	9
I	0	1	0	1	0	2	0	0	0
R	8	7	7	2	9	3	8	3	1
IDO/OSI									
S	8	3	6	4	3	9	4	8	10
I	1	3	3	2	1	1	1	1	0
R	1	4	1	4	6	0	5	1	0
MOBA									
S	6	7	5	3	1	8	7	7	10
I	1	1	2	3	2	1	2	1	0
R	3	2	3	4	7	1	1	2	0
TOTAL(%)									
S	42	36	39	20	21	70	33	62	93
I	7	9	14	13	4	17	6	9	2
R	51	55	47	67	75	13	61	29	5

Table.2 Antibiotics resistance pattern of *Escherichia coli* isolated from cow dungs across 10 local government areas of Ekiti State, Nigeria

LG N=10	Antibiotics AMX	COT	NIT	GEN	NAL	OFL	AUG	TET
ADO								
S	0	0	0	7	0	7	0	0
I	0	0	0	2	0	2	0	1
R	10	10	10	1	10	1	10	9
EFON								
S	1	0	2	10	1	9	1	1
I	0	0	0	0	0	1	0	0
R	9	10	8	0	9	0	9	9
EKITI WEST								
S	2	2	2	9	4	6	2	3
I	0	1	0	1	1	4	0	0
R	8	7	8	0	5	0	8	7
EKITI SWEST								
S	0	0	0	8	0	8	0	0
I	0	0	0	2	0	2	0	0
R	10	10	10	0	10	0	10	10
IKERE								
S	0	0	1	9	2	8	2	2
I	0	0	0	1	0	2	0	0
R	10	10	9	0	8	0	8	8
IKOLE								
S	0	0	0	7	1	10	0	0
I	0	0	1	3	2	0	0	2
R	10	10	9	0	7	0	10	8
IJERO								
S	2	2	3	7	3	8	4	3
I	1	1	0	2	1	2	0	0
R	7	7	7	1	6	0	6	7
IDO/OSI								
S	0	0	0	9	4	6	2	3
I	0	0	0	0	1	4	0	0
R	10	10	10	1	5	0	8	7
IRE/IFELODUN								
S	0	2	2	7	2	8	2	1
I	0	0	0	3	1	2	1	0
R	10	8	8	0	7	0	7	9
MOBA								
S	1	2	1	7	1	9	2	0
I	0	1	1	1	1	1	1	0
R	9	7	8	2	8	0	7	10
TOTAL(%)								
S	6	8	11	80	18	79	15	13
I	1	3	2	15	7	20	2	3
R	93	89	87	5	75	1	83	84

The high incidence of resistance of both organism to augmentin, tetracycline, amoxicillin and cotrimoxazole may result from acquired resistance determinants for theses antibiotics. However, the incidence of gentamicin resistance for both organisms was low; an indication of a high potency of the antibiotics (Akond *et al.,* 2009; Hershberger *et al.,* 2005). This was contrary to the findings of Daini and Adesemowo. (2008) who reported 88% resistance of *E. coli* to gentamicin and determined the antimicrobial susceptibility pattern and R plasmids of clinical strains of *E. coli*.

Indiscriminate administration of antibiotics to cattle to prevent diseases and as growth supplement by herd's men may have heightened the antibiotics resistance of *Enterococcus faecalis* and *Escherichia coli* in this study. The multiple resistance strains of both organisms could be disseminated into the environment and transmitted to man through consumption of beef or even faecal contamination of streams/rivers that the various community in the study uses for drinking and other domestic purposes (Van der Boogard and Stobberinh, 2000; Apun *et al.,* 2008). This process may further compromise the use of drugs in hospitals and may also result in antibiotics treatment failure (Davies, 1994). Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections predominantly caused by *E. coli* which are major source of illness, death and increased healthcare costs (Gupta *et al.,* 2001).

In developing countries, reducing the infection and diseases caused by these organisms require observance of good personal hygiene especially among herd's men. The herd's men and veterinarian should work closely when antibiotics therapy is needed and both must also work towards ensuring a safe food supply for the consumers.

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