academicJournals

Vol. 7(37), pp. 4572-4575, 13 September, 2013
DOI: 10.5897/AJMR2013.6028
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antibiotic resistance in *Escherichia coli* isolated from healthy cattle at a major cattle market in Ibadan, Oyo State, South Western, Nigeria

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Accepted 6 September, 2013

Isolation of multidrug resistant *Escherichia coli* from diseased livestock is becoming rampart from samples submitted for disease diagnostic purposes in some Tertiary Veterinary Teaching Hospitals in Nigeria. In order to evaluate the possible roles of commensal *E. coli* from apparently healthy animals in the epidemiology of drug resistant pathogens, antibiotic resistance status of 240 *E. coli* isolated from 300 rectal swab from apparently healthy cattle from a major cattle market in Ibadan, Oyo State, South West Nigeria (a location very close to two of the Veterinary Teaching Hospitals), were grown aerobically at breakpoint concentration for ciprofloxacin, cefepime, chloramphenicol, tetracycline, ampicillin, kanamycin, streptomycin and nalidixic acid (all obtained from SIGMA-ALDRICH) according to standard method by Clinical Laboratory Standards Institute. A very high level of resistance was observed in all the antibiotics studied, with the highest resistance of 97% for kanamycin and 96.7, 95.8, 73.8, 72.5, 61.7, 56.3 and 51.3% for ampicillin, chloramphenicol, tetracycline, cefepime, nalidixic acid, streptomycin and ciprofloxacin, respectively. The high resistance observed in the commensal *E. coli* could be attributed to antibiotic selective pressure due to often abuse/misuse of the drugs on the cattle. The findings in this work are of public health importance because these multidrug resistant *E. coli* can transmit resistance to other pathogenic bacteria of livestock and human pathogen.

Key words: Antimicrobial, resistance, healthy cattle, isolated.

INTRODUCTION

Antibiotic resistance among microorganisms is a major problem, both in human and livestock industry. And the problem is usually attributed to unregulated and inappropriate use of antibiotics (Witte, 1998; Lawson, 2008). It has been severally reported that commensal *Escherichia coli* isolates from healthy animals like cattle, swine as well as from humans play significant roles in the perpetration of drug resistant pathogens and subsequent infections (Hunter et al., 1993; Shanahan et al., 1995; Sunde and Sørum, 1999; Gulay et al., 2000; Kozak et al., 2009; Tian et al., 2012).

In Nigeria livestock industry, the problem of occurrence of multidrug resistant *E. coli* is becoming very rampart, because they are often encountered in routine diagnoses of disease conditions from livestock brought for confirmatory diagnosis in microbiology diagnostic units of some Tertiary Veterinary Teaching Hospitals in Nigeria (unpublished data). Earlier in Nigeria, Ogunleye et al. (2008), reported nineteen different multidrug resistant patterns to commonly available antibiotic in *E. coli*

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isolated from some diseased poultry samples from eleven poultry farms in Abeokuta, Ogun State, Nigeria. The 39 *E. coli* were isolated from various organs like liver, lungs, kidneys, ovary, intestine and colo-rectum of birds that died of septicaemic conditions. Each of the *E. coli* isolates studied were resistant to between 5 and 12 commonly used antibiotics such as nitrofurantoin, cefuroxime, norfloxacin, cotrimoxazole, nalidixic acid, chloramphenicol, ampicilin, ofloxacin, penincilin G, amoxylin, cloxacilin and ciprofloxacin(Ogunleye et al., 2008).

More recently, Ajayi et al. (2011) in a study, determined the antibiotic susceptibility patterns of commensal *E. coli* from feaces of apparently healthy cattle in Ado Ekiti, Ekiti State Nigeria from ready to slaughter cattle. They observed 74 different multiple antibiotic resistance patterns in the commonly used antibiotics, and 81% (851/1051) of the isolated *E. coli* showed resistance to at least 3 of the eight antibiotics tested, while 20 isolates showed resistance to all the eight antibiotics namely: ampicillin, cortrimoxazole, gentamicin, nalidixic acid, nitrofurantoin, colistin, streptomycin and tetracycline (Ajayi et al., 2011). Subsequently, from a group of 500 *E. coli* isolates tested for susceptibilities to fluoroquinolones (norfloxacin, levofloxacin, pefloxacin, ofloxacin and ciprofloxacin) and cephalosporin group of drugs (ceftazidime, cefoxitin, ceftriaxone and aztreonam); the isolates were more sensitive to the cephalosporin group of drugs than to the fluoroquinolones based drugs (Ajayi et al., 2011).

In another study from the South Eastern part of Nigeria, Nsofor and Iroegbu (2012), reported their observation on the antibiotic profiles of *E. coli* isolated from apparently healthy livestock like cattle, goat, poultry and swine. They observed 42 different antibiotic resistance profiles, with each isolates showing resistance to at least 4 or more of the antibiotics tested including: ampicilin, cortrimoxazole, cephalothin, streptomycin, nitrofurantoin, tetracycline, chloramphenicol, amoxicillin- clavulanic acid, cefpirome, cefpodoxime, cefotaxime, ceftriaxone, cefoxitin, nalidixic acid and gentamicin (Nsofor and Iroegbu, 2012). The current study was carried out to evaluate the current status of antibiotic resistance to commonly used antibiotics among the *E. coli* isolates from apparently healthy cattle at a major cattle market, located in Ibadan, Oyo State Nigeria: where a large number of cities and towns in the South Western Nigeria States purchase cattle for slaughter for human consumptions. The public health implications of the findings were discussed.

MATERIALS AND METHODS

Sample collection/location

A total of 300 rectal swabs were sampled from apparently healthy cattle at Akinyele cattle market Ibadan, Oyo State, Nigeria. All the cattle sampled belonged to three indigenous breeds namely: 'Sokoto Gudali', 'White Fulani' and 'Kuri', and all fall between two and five years. The cattle market is one of the biggest in the South Western Nigeria. Cattle sold in the market were usually brought from the Northern States of Nigeria such as Borno, Sokoto, Kaduna, Kano, Niger and Kebbi States and sometimes from the neighboring West African countries like Niger Republic, Chad Republic and occasionally from Cameroon. Antibiotics usage is a common practice while transporting the cattle to the site and also during the transient period between the arrival of the cattle to the market and the time of sales to cattle butchers and other end users for slaughter. Some of the antibiotics reported to be commonly used both in transit as well as during the time lag between the arrival of the cattle at the market and the time of sales for slaughter include: oxytetracycline (short acting) injectable, procaine - penicillin injectable, streptomycin injectable and occasionally penincillin-streptomycin injectable. The samples were collected between March and June, 2012.

Bacteriological analysis

Rectal swab samples were streaked on MacConkey agar and incubated aerobically at 37°C for 24 to 48 h. All the isolates that fermented lactose were sub cultured onto Eosin Methylene Blue agar (EMB) and incubated at 37°C for 24 to 48 h. All the isolates that produced typical metallic - sheen on EMB were further subjected to morphological and biochemical analysis by standard methods (Barrow and Felthams, 1993; Garcia and Isenberg, 2007). Antibiotic resistant pattern of two hundred and forty *E. coli* isolated from the samples were grown aerobically in breakpoint concentration of 8 µg/ml for ciprofloxacin, and 32 µg/ml for each of: cefepime, chloramphenicol, tetracycline, ampicillin, kanamycin, streptomycin and nalidixic acid (all obtained from SIGMA-ALDRICH) according to standard method (CLSI, 2009). Resistance was ascribed if flocculent growth was observed after 16 h of aerobic growth at 37°C.

RESULTS

E. coli were isolated in 240/300(80%) of the rectal swabs processed. As shown in Table 1 and Figure 1, the highest resistance of 97% at the antibiotic breakpoint concentration was observed in kanamycin, followed closely by 96.7 and 95.8% for ampicillin and chloramphenicol, respectively. The following percentage resistance of 73.8, 72.5, 61.7, 56.3 and 51.3% were observed for tetracycline, cefepime, nalidixic acid, streptomycin and ciprofloxacin, respectively. Table 1 and Figure 2 show the sensitivity pattern and proportions of the tested *E. coli* to the antibiotic. Of all the antibiotics tested, ciprofloxacin was the most sensitive with 48.7% sensitivity, while kanamycin with 3.0% sensitivity was the least sensitive.

DISCUSSION

In recent times, multidrug resistant *E. coli* are often isolated from disease conditions from livestock during routine diagnosis in some diagnostic units of some Tertiary Veterinary Teaching Hospitals in Nigeria (unpublished data). The possible roles of multidrug resistant *E. coli* from healthy animals in the transfer of drug resistance to pathogenic bacteria in animal and humans have been acknowledged (Gulay et al., 2000; Kozak et al., 2009; Tian et al., 2012). The occurrence of high level of antibiotic resistance obtained from the *E. coli* from healthy cattle studied gives an indication of the possible roles

Table 1. Percentage sensitivity and resistant patterns of *E. coli* from cattle to eight antibiotics using antibiotics breakpoint method.

Antibiotic	Resistant at breakpoint concentration.	Sensitive at breakpoint concentration.
Cefepime	174/240(72.5%)	66/240(27.5%)
Ciprofloxacin	123/240(51.3%)	117/240(48.7%)
Streptomycin	135/240(56.3%)	105/240(43.7%)
Tetracycline	177/240(73.8%)	63/240(26.2%)
Ampicillin	232/240(96.7%)	7/240(3.3%)
Chloramphenicol	230/240(95.8%)	10/240(4.2%)
Nalidixic acid	148/240(61.7%)	92/240(38.3%)
Kanamycin	233/240(97.0%)	7/240(3.0%)

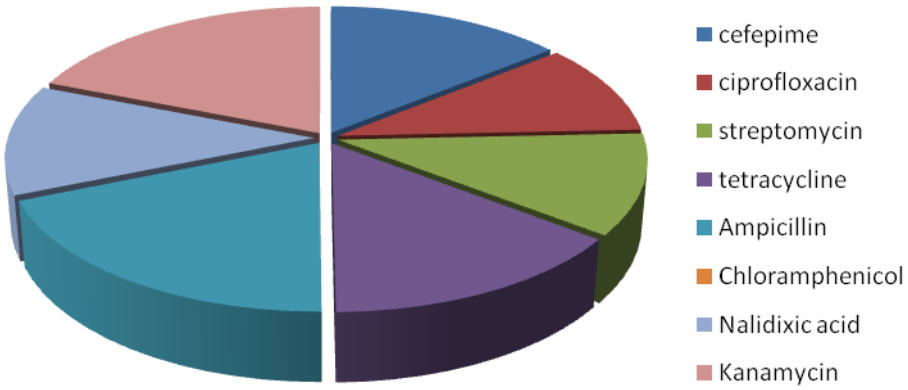

Figure 1. Proportions of the percentage resistance of the *E. coli* for each of the antibiotics studied.

of these bacteria in the transfer of the commonly observed resistance among pathogenic *E. coli* from livestock in Nigeria. The high level of drug resistance obtained from *E. coli* isolated from apparently healthy cattle in this study is comparable with the findings of other workers in *E. coli* isolates from healthy livestock from some other parts and locations in Nigeria. For instance, using a disk diffusion method, Ajayi et al. (2011) reported 67.6% (710/1051) resistance for streptomycin among *E. coli* isolated from apparently healthy cattle ready for slaughter in Ekiti State, Nigeria, and 62.5%(n = 80) from livestock in the South Eastern Nigeria (Nsofor and Iroegbu, 2012), while in the current work using antibiotic breakpoint method, 56.3% (135/240) resistance was obtained for streptomycin. The percentage resistance in the current work for tetracycline and ampicillin were also comparable to the findings of Ajayi et al. (2011) and that of Nsofor and Iroegbu (2012). For tetracycline, there was 73.8% (177/240) resistance in the current work; 64.3% (676/1051) for Ajayi et al. (2011), 68.8% (n = 80) for Nsofor and Iroegbu (2012). For ampicillin, there was 96.7% (232/240) resistance in the current work, 85.3% (896/1051) in Ajayi et al. (2011) and 85% (n = 80) from the work of Nsofor and Iroegbu (2012).

The highest level of resistance in the commensal *E. coli* studied by Ajayi et al. (2011) in cattle at Ekiti State was 88.1% (892/1051) in gentamicin. However, gentamicin was not part of the antibiotics used for the breakpoint determination, but among the antibiotic studied, kanamycin produced the highest resistance of 97% followed closely by ampicillin 96.7% and chloramphenicol 95.8%. And unlike the work of Ajayi et al. (2011), where nalidixic acid produced the least percentage of resistance of 9% (95/1051), a higher resistance of 61.7% (148/240) was recorded at the breakpoint concentration for the *E. coli* isolates in this study. The high level of resistance obtained for the representative antibiotics of the fluoroquinolone (ciprofloxacin) 51.3% and the 3rd generation cephalosporin (cefepime) 72.5% are also noteworthy because of their importance as drug of choice in treating life threatening bacterial infections in livestock and human. Indiscriminate use of antibiotics without cogent indication for its use is a common practice while transporting the cattle from the Northern part of the country and from the neighboring West African countries to this cattle market. Through interaction with the cattle dealers, it was also discovered that they were fond of using certain antibiotics routinely. For example, streptomycin

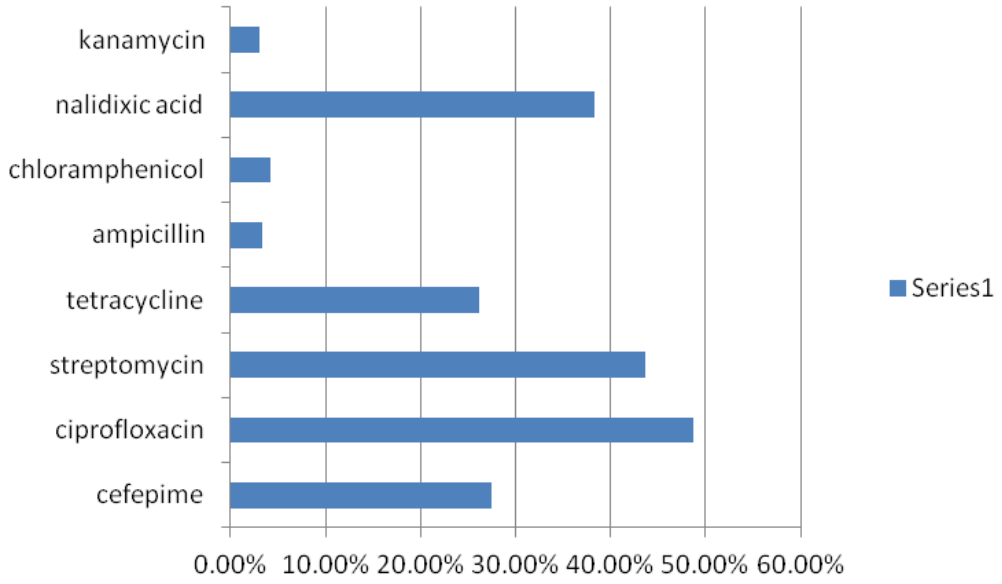

Figure 2. Percentage resistance of the *E. coli* for each of the antibiotics studied.

is often used without prescriptions any time they found any of the animals coughing, oxytetracycline during cold seasons and penicillin-streptomycin for all manner of infections, all these without careful attention to administration of correct dose/dosages nor based on veterinary prescriptions. These unwholesome practices must have contributed to the presence of high level of the drug resistant *E. coli* isolated from the healthy cattle studied due to antibiotics selective pressure. The large percentages of the bacteria studied is not susceptible at the respective breakpoints of the studied antibiotics which is an indication of their tolerance to the antibiotics and possibility of difficulty in treating possible infections by these organisms by the antibiotics concerned (Rodriguez-Bano et al., 2011). Since there is the possibility of acquisition of drug resistance mechanism through interbacterial transfer (Falagas et al., 2012), the current work thus underscores the public health risk of the possible transfer of this high drug resistant trait from this apparently non-pathogenic *E. coli* to pathogenic animal pathogens as well as to humans.

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