

Full Length Research Paper

Plasmid profile of antibiotic resistant *Escherichia coli* isolated from domestic animals in South-East Nigeria

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Plasmid profiling is among the methods used to determine and characterize antibiotic resistance traits in bacteria. In this study, a total of 80 *Escherichia coli* isolate from four domestic livestock comprising cattle, goats, poultry and swine in three states of South East Nigeria were screened for antibiotic susceptibility and plasmid profiles. The isolates were tested against 14 antibiotics using the disc diffusion method while plasmid DNA was extracted using the alkaline SDS method and separated by agarose gel electrophoresis. A total of 42 different antibiotic resistance profiles were observed, with each isolate showing resistance to at least four or more drugs tested. Plasmids of different sizes were detected in the isolates. Isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range of 1 to 120 KB. Very high resistance levels (>75%) were detected against Ampicillin, Cotrimoxazole, Cephalothin and Streptomycin, while Nalidixic acid and Gentamycin recorded the least resistance levels of 16.3 and 12.5% respectively among the isolates.

Key word: Plasmid profile, *Escherichia coli*, Nigeria.

INTRODUCTION

Antibiotics have helped in reducing diseases in animal husbandry; however, there is a growing awareness of public health concerns associated with the use of antibiotics (Van der-Auwera et al., 2009). Carraminana et al. (2004) reported that 40% of the antibiotics produced in the United States were used in stock feeds. The widespread use of various antibiotics for treating animal infections has created antibiotic resistant bacterial strains (Tivendale et al., 2009; Sackey et al., 2001). Bacterial isolates obtained by Carraminana et al. (2004) from a poultry slaughter house in Spain had high percentages of resistance to many antibiotics such as neomycin (53.4%), tetracycline (21.8%), and streptomycin (11.3%). Nsofor and Iroegbu (2012) reported very high frequency of resistance to Ampicillin (85%), Cotrimoxazole (90%), Cephalothin (90%) and Streptomycin (77.5%) in *Escherichia coli* strains isolated from animals in Nigeria. Multiple antibiotic resistant strains can be transported

from animals to humans by food (Whitworth et al., 2008; Daniels et al., 2009; Nsofor and Iroegbu, 2013).

Animal feces are potential source of antibiotic resistant bacteria. If released into the environment, resistant strains may contaminate water and food sources and can be a potential threat to human health (Roy et al., 2009). *E. coli* strains isolated from sewage treatment plants were reported to be resistant to various antibiotics (Reinthal et al., 2003).

Plasmids are major mechanism for the spread of antibiotic resistant genes in bacterial populations (Fang et al., 2008). Conjugation occurs by F-plasmids that can transfer genes encoded for multiple resistance and mobilize other non-conjugative plasmids to host cells. Multiple resistance genes are harbored on R-plasmids some of which are conjugative (Pitout et al., 2009). *E. coli* have been reported to transfer the antibiotic resistant genes to enteric pathogenic and normal flora bacteria,

Table 1. The distribution of plasmids according to hosts.

Plasmid size (KB)	Frequency of distribution according to source			
	Cattle N=30 (%)	Goat N=29 (%)	Poultry N=17(%)	Pig N=24 (%)
1.0	03(10)	02(6.9)	01(5.9)	00
1.4	00	00	00	03(12.5)
1.5	02(6.7)	02(6.9)	1(5.9)	01(4.2)
1.8	00	00	00	00
2.0	01(3.3)	01(3.4)	01(5.9)	01(4.2)
2.5	05(16.7)	07(24.1)	02(11.8)	02(8.3)
3.0	01(3.3)	02(6.9)	02(5.9)	01(4.2)
4.0	03(10)	02(6.9)	00	00
4.5	00	01(3.4)	00	00
5.0	01(3.3)	01(3.4)	01(5.9)	04(16.7)
7.0	00	00	01(5.9)	02(8.3)
10.0	01(3.3)	01(3.4)	00	00
20.0	00	01(3.4)	00	00
55.0	00	01(3.4)	02(11.8)	00
95.0	04(13.3)	05(17.2)	01(5.9)	02(8.3)
120	09(30.0)	05(17.2)	06(35.3)	05(20.8)

N, number of plasmids.

such as, *Salmonella* spp and *Proteus* spp (Yoon and Hovde, 2008). The objective of this study was to investigate plasmid profile of antibiotic resistant *E. coli* isolated from domestic live stock in South East Nigeria.

MATERIALS AND METHODS

Fresh fecal droppings were randomly collected from goats, cattle, pigs, and chicken; and care was taken to avoid collecting more than one fecal sample per individual animal. *E. coli* strains were isolated and identified by standard microbiological methods (Cheesbrough, 2000). The isolates were screened for antibiotic susceptibility using the disc diffusion method on Mueller-Hinton agar (Oxoid, England). Fourteen antibiotics were used: Ampicillin (10 µg), Amoxicillin/clavulanic acid (20/10 µg), Cefoxitin (30 µg), Cefpodoxime (10 µg), Cefpirome (30 µg), Ceftriaxone (30 µg), and Cefotaxime (30 µg), Cephalothin (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Nalidixic acid (30 µg), Nitrofurantoin (30 µg), Streptomycin (10 µg), Sulfamethoxazole-trimethoprim (10 µg) and Tetracycline (30 µg), (Oxoid, England). Inhibition zone diameters were measured after 24 h of incubation at 37°C and were interpreted using the breakpoints of the Clinical Laboratory Standard Institute (CLSI, 2006). A standard *E. coli* (ATCC 25922) was used as a control. No antibiotic was included in the EMB agar plates used for the cultivation. All the animals included in this study were (at the time of specimen collection) not showing any sign of ill-health. The cattle and goat specimens came from the heard at Obinze, Owerri, Imo State while the Madonna University Poultry, Anambra State was the source of poultry specimens. The specimens from swine came from a farm located at the Ogorhil area of Abia state. There was no documented evidence of antibiotics use in the farms from which the specimens were collected.

Plasmids were extracted using the alkaline SDS method (Kado and Liu, 1981). The extracted plasmids were separated by agarose gel electrophoresis for their profiling. Gel electrophoresis was carried out with 0.8% agarose in 1x TAE buffer (121 Tris-Base,

22.55 glacial acetic acid and 50 ml of 0.5M EDTA pH 8.0) on a horizontal gel apparatus at 100 V/cm² for 3 h. The BAC-Tracker Super coiled DNA (Invitrogen) and 1KB plus DNA ladders (Invitrogen) were included for the estimation of plasmid sizes. The gel was stained with 5 µl of 10 mg/ml of ethidium bromide, visualized by UV trans-illuminator (Fisher Scientific) and photographs were taken with gel imager (Alpha Innotech Corporation, San Leandro, CA, USA).

RESULTS

Eighty *E. coli* strains were isolated from the various domestic livestock and tested against 14 antibiotics. The isolates show resistance rates of 85% to Ampicillin; 90% to Cotrimoxazole; 90% to Cephalothin; 77.5% to Streptomycin; 62.5% to Nitrofurantoin; 68.8% to Tetracycline; 55% to Chloramphenicol; 56.3% to Amoxicillin clavulanic acid; 58.8% to Cefpirome; 47.5% to Cefpodoxime; 43.8% to Cefotaxime; 22.5% to Ceftriaxone; 18.8% to Cefoxitin; 16.3% to Nalidixic acid and 12.5% to Gentamycin. All isolates were resistant to at least four or more antibiotics.

Plasmids analyses revealed that all the isolates harbored one or more plasmids with molecular weight in the range of 1 to 120 KB. Of the 100 plasmids detected in the isolates, 42 different profiles were recorded, 9 in pigs, 13 in goats, 10 in cattle and poultry, respectively (Table 1). The 1.5, 2, 2.5, 3, 5, 95 and 120 KB plasmids were detected across the animal hosts. The 55 KB plasmid occurred at the rate of 3.4 and 11.8% in goats and poultry isolates respectively. The only 20 KB plasmid detected in this study came from goat specimen, while isolates from pig harbored the three 1.4 KB plasmid

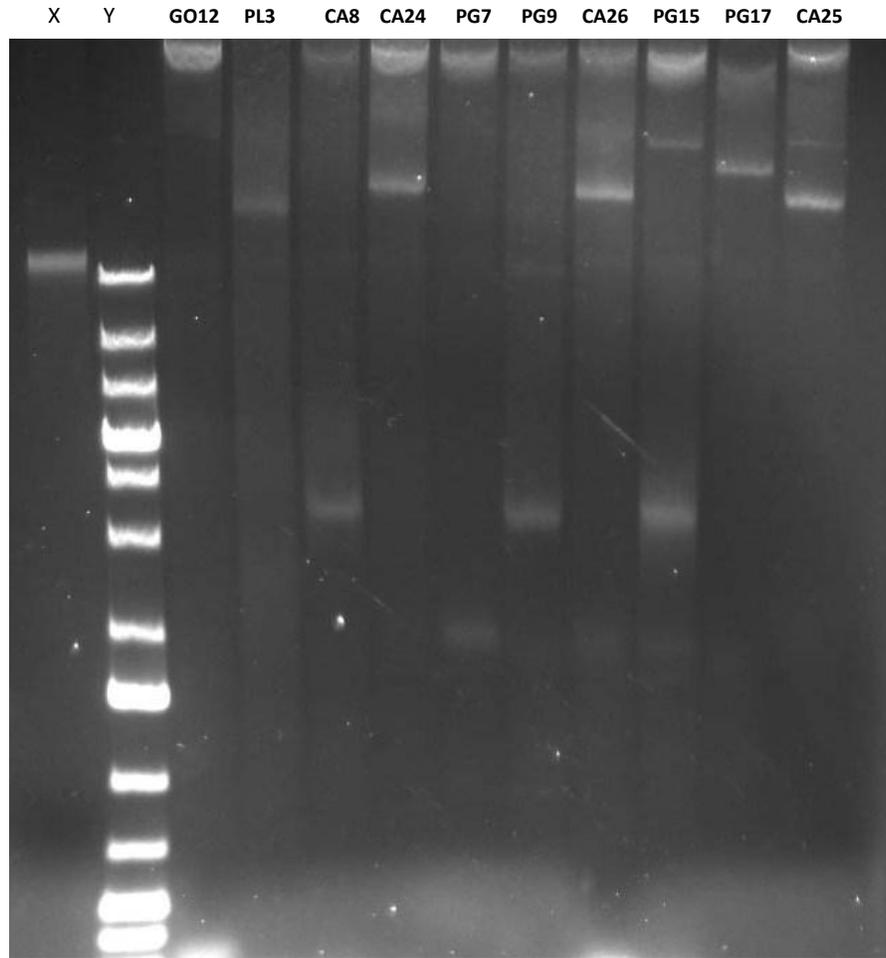


Figure 1. The Plasmid Profile of first group of *E. coli* Isolates from Animal Specimens. X, Bac-tracker super coiled DNA Ladder; Y, 1KB plus DNA Ladder; Z, 120KB Plasmid positive control.

observed in this study. The 120 KB plasmid was most frequent in three, out of four animals sampled; 20.8% in pig, 30% in cattle and 35.3% in poultry, respectively. The most frequently isolated plasmid in goat isolates was the 2.5KB plasmid with frequency of 24.1%. Figures 1 to 6 below show the detailed gel images of some of the plasmid profiles.

DISCUSSION

Multiple resistances were reported to be more common than resistance to single antibiotics. It was also reported that a high percentage of *E. coli* (88.2%) isolated from Swine feces were resistant to one or more antibiotics (Nsofor and Iroegbu, 2012). In this study, all of the isolates were resistant to at least four or more antibiotics. Tetracycline resistance was also observed among *E. coli* isolates and has been frequently reported in poultry products. Tabatabaei and Nasirian (2003) reported that

94% of *E. coli* isolates from chickens were resistant to tetracycline and 46% were resistant to ampicillin. Their findings are similar to our results, tetracycline is one of the broad-spectrum antibiotics available in feed supplements, and its improper use led to the development of multiple antibiotic resistances.

The high rates of resistance found in this study can be explained by the wide use of antibiotics in Nigeria for prophylaxis and for treatment in poultry farms. The improper and unnecessary use of antimicrobial drugs in man also promotes development of resistant strains with R-plasmids. Nsofor and Iroegbu (2013) reported that both pathogenic and non-pathogenic strains of *E. coli* resistant to drugs may be transported from animals to humans via food. Such strains act as an important source for *in vivo* transmission of R-plasmids to drug sensitive strains in the animal intestine mainly through conjugation.

A great similarity between the plasmids of Enterobacteriaceae isolated from animals and humans has been observed. Other workers reports that, transmission

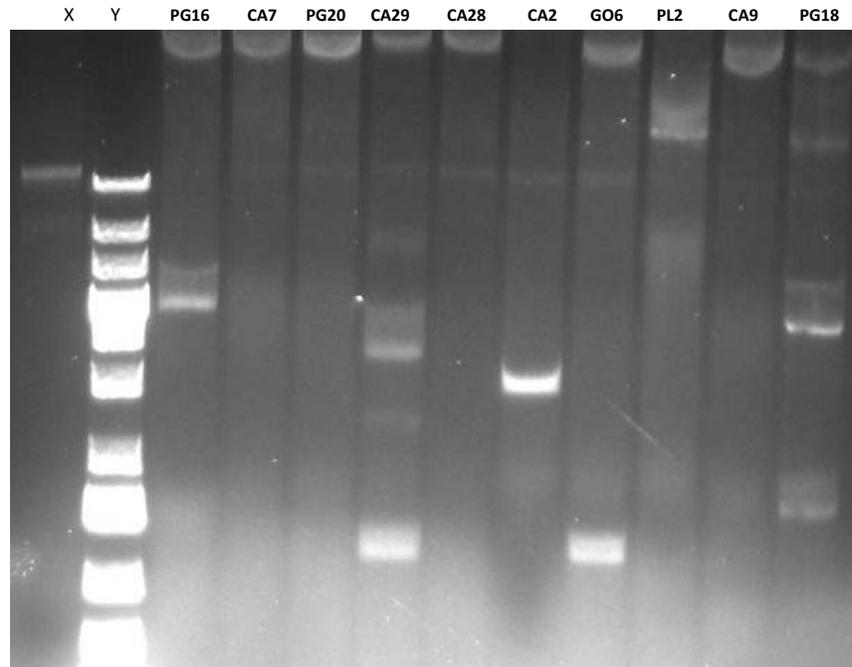


Figure 2. The Plasmid Profile of second group of *E. coli* Isolates from Animal Specimens. X, Bac-tracker super coiled DNA Ladder; Y, 1 KB plus DNA Ladder; Z, 120 KB Plasmid positive control.

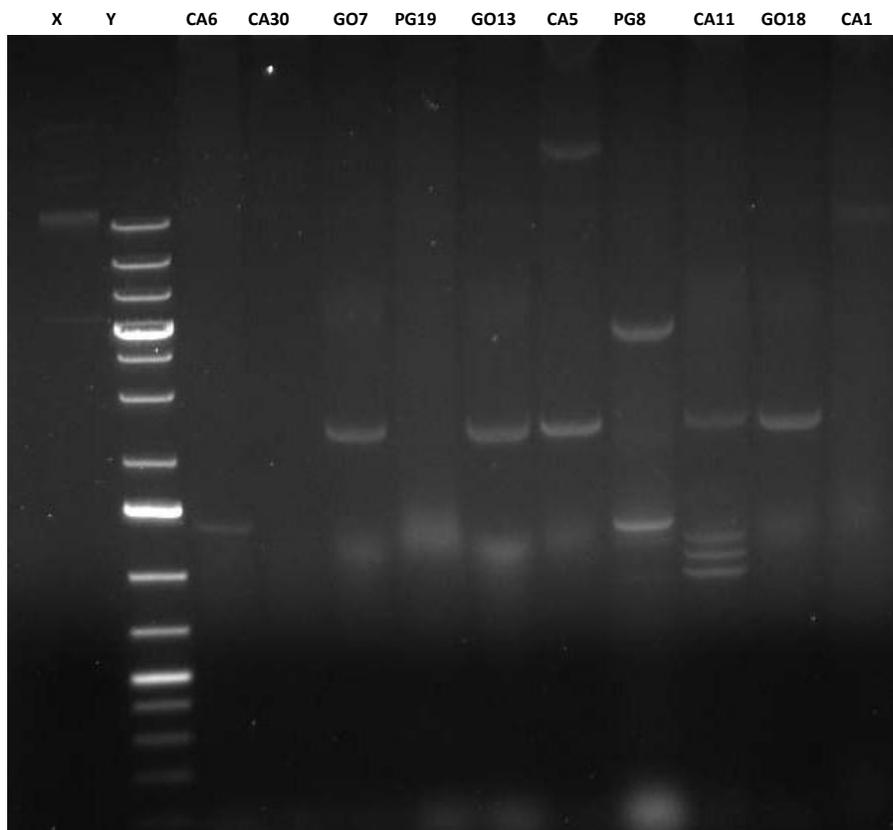


Figure 3. The Plasmid Profile of Third group of *E. coli* Isolates from Animal Specimens. . X, Bac-tracker super coiled DNA Ladder; Y, 1 KB plus DNA Ladder; Z, 120 KB Plasmid positive control.

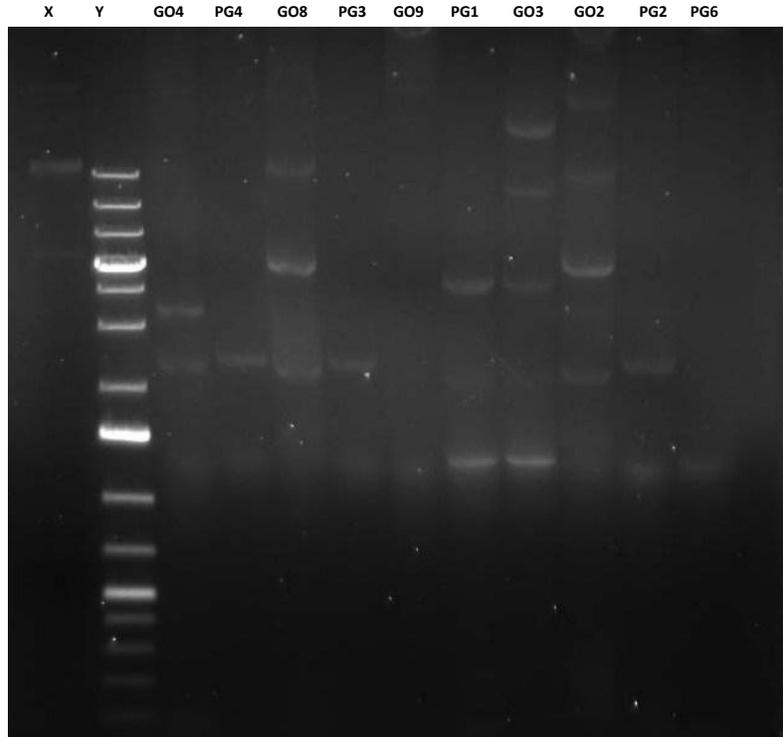


Figure 4. The Plasmid profile of the fourth group of *E. coli* Isolates from Animal Specimens. X, Bac-tracker super coiled DNA Ladder, Y=1KB plus DNA Ladder, Z=120KB Plasmid positive control.

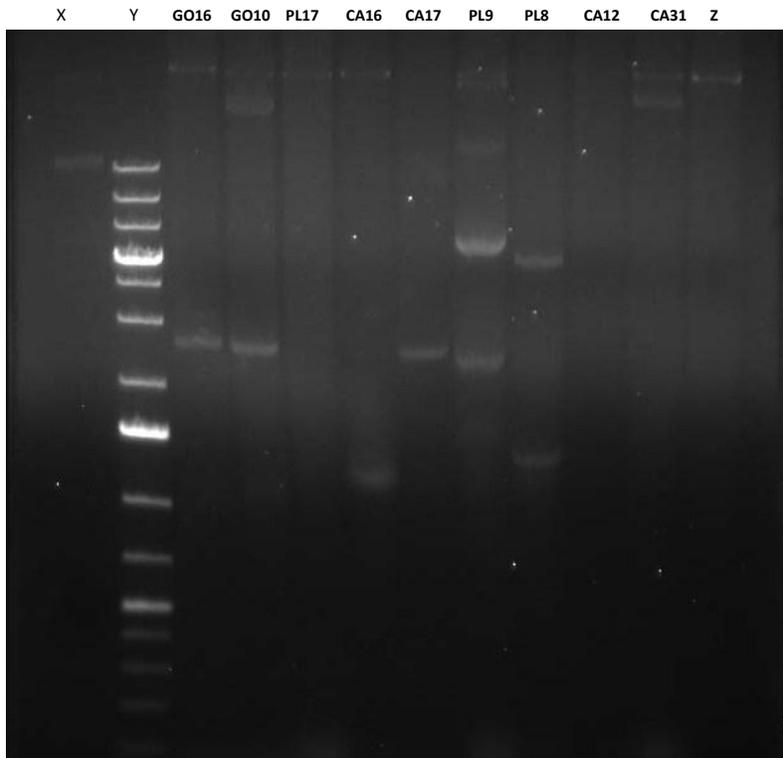


Figure 5. The plasmid profile of the fifth group of *E. coli* Isolates from animal specimens. X, Bac-tracker super coiled DNA Ladder; Y, 1KB plus DNA Ladder; Z, 120 KB Plasmid positive control.

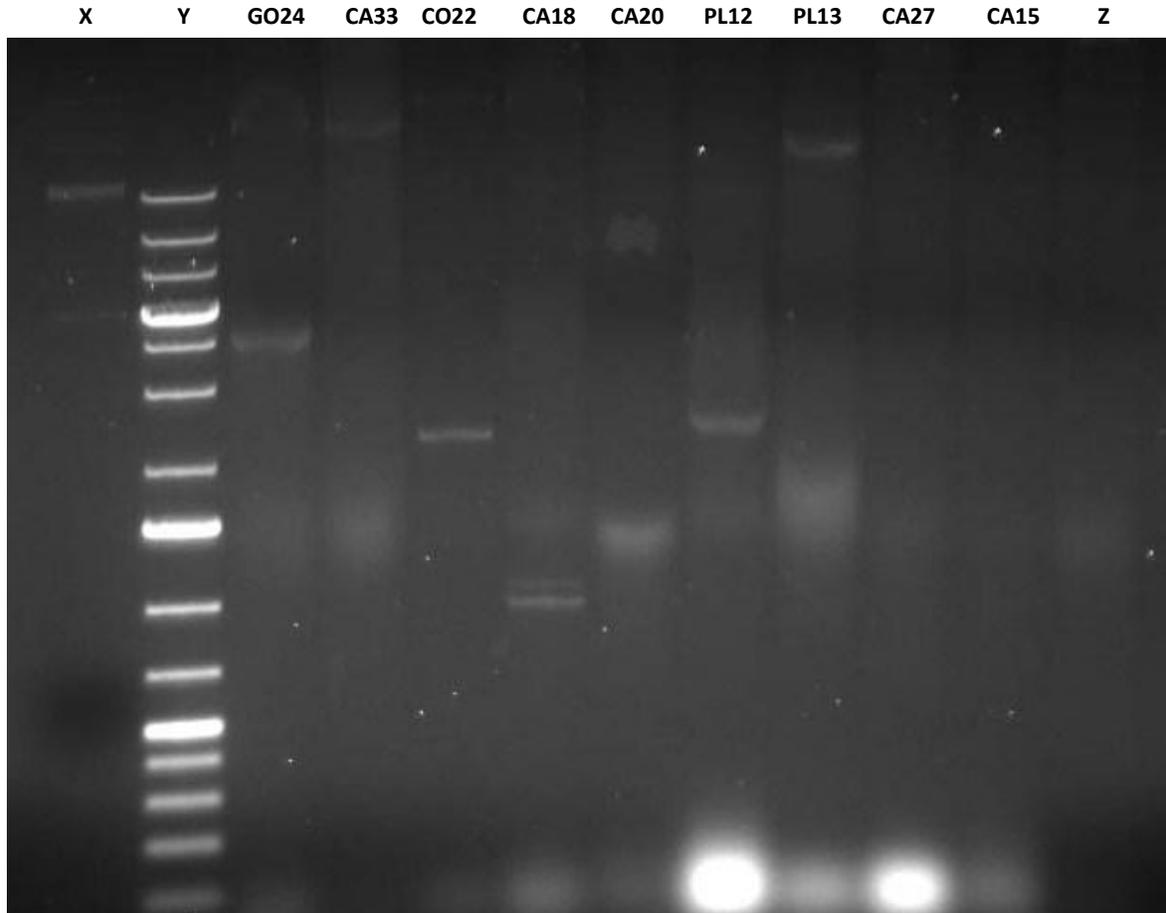


Figure 6. The plasmid profile of the sixth group of *E. coli* isolates from animal specimens. X, Bac-tracker super coiled DNA Ladder; Y, 1 KB plus DNA Ladder; Z, 120 KB plasmid positive control.

of resistance plasmids of *E. coli* from animals to humans commonly occurs. The plasmid DNA analysis of the strains shows that the size of the plasmids varied. Although, some strains were resistant to only four antibiotics, they had more than one plasmid while others containing 1 or 2 plasmids were resistant to a large number of antibiotics. Içgen et al. (2002) reported similar findings. In their study, plasmid DNA analysis of the 28 *Salmonella* strains showed that the size of the plasmid DNA ranged from 3.1 to 32 kb. From their report, most strains associated with non-human sources were found to harbor larger plasmids while most human strains have relatively much smaller plasmids with sizes 10.8, 9, 4.7 and 6.2 kb pairs; yet they were resistant to a larger number of antibiotics.

This suggests that not all antibiotic resistance genes are located in plasmids. Some of the genes conferring resistance may be located on bacterial chromosome. Aja et al. (2002) in their study of *Vibrio* strains isolated from cultured shrimps showed that some strains were resistant to four antibiotics, others were resistant to two antibiotics and all contained one plasmid of 21.2 kb pair. They

suggested that resistance to antibiotics could be encoded in some strains in plasmids and in others in the chromosomes. It is well established that antibiotic pressure supports resistant strains and eliminates sensitive strains. The more antibiotics used, the more the elimination of the sensitive strains thereby, allowing resistant strains to dominate. It is also true that resistant strains are outcompeted by sensitive strains when antibiotic pressure is removed from the environment. Thus, steps must be taken to control the overuse of antibiotics in Nigeria, as well as in other developing countries.

In conclusion, it is of significant public health concern that multidrug-resistant commensal *E. coli* strains may constitute a potential reservoir of resistance plasmids that could be transferred to pathogenic bacteria. The findings of this study provide evidence to support studies that suggest the existence of a reservoir of antibiotic resistance genes. Infections with multidrug-resistant pathogens limit the options available to treat infectious disease of animals and humans. The high prevalence of multidrug-resistant *E. coli* observed in this study suggests the need for improved education and communication on

the issue of antibiotic used in veterinary medicine. Finally, we hope that our results will lead to further research on how drugs are used in veterinary medicine and subsequently guide therapy for *E. coli* infections in Nigeria.

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