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Detection of antibiotic resistance genes of *Escherichia coli* from domestic livestock in south east Nigeria with DNA microarray

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DNA microarray was developed for detection of up to 90 antibiotic resistance genes in *Escherichia coli* by hybridization. Each antibiotic resistance gene was represented by two specific oligonucleotides chosen from consensus sequences of gene families. A total of 203 oligonucleotides (50-100 base) were spotted onto the microarray. The sequence identity of each gene was compared with GenBank sequences, biotin was used as the positive control and 16s rRNA as orientation. Of the 40 *E. coli* isolates analyzed in this study, 37 were identified as having, at least, one antibiotic resistance gene. Among the different antibiotic resistance genes detected, *bla-CMY-2* and *strA* were the most prevalent occurring in 28 (70%) of the isolates, respectively. Other common genes included were *TEM1* 11(27.5%), *Sul2* 14 (35%) and *TetA* 21(52.5%). The microarray genotyping corresponded with the phenotype of the strains. The disposable microarray presents the advantage of rapidly screening bacteria for the pre-sence of known antibiotic resistance genes. This technology has a large potential for applications in basic research, food safety, and surveillance programs for antimicrobial resistance.

Key words: DNA microarray, antibiotic resistance, *Escherichia coli*.

INTRODUCTION

During the past decades, the worldwide use of antibiotics in animal husbandry for purposes of prophylaxis, chemotherapy and growth promotion has created enormous pressure for the selection of antibiotic resistance among bacteria (Vincent et al., 2005). Today, there is increasing concern about the severity of antibiotics resistance in *Escherichia coli*, which is an important reservoir of antibiotic resistance genes; many other enteric pathogens and commensal bacteria may also play a role as reservoirs for antibiotics genes (Greg et al., 2010; Ma et al., 2007). It is therefore important to follow the evolution of antibiotic resistance in the bacterial population in order to prevent and repress the emergence of multidrug-resistant strains of those bacteria that can still be treated with antibiotics.

The disc diffusion assay technique is commonly used to determine the resistance of pathogenic or commensal bacteria because of its simplicity and because it provides information that is useful in prescribing appropriate antibiotics. Phenotypic testing such as disc diffusion assay technique, however, will not detect ''silent'' antibiotics resistance genes that might be expressed *in vivo* or disseminated to other bacteria (Frye et al., 2006; Nsofor and Iroegbu 2012, 2013). Molecular testing methods offer similar information more quickly and provides for more discriminatory information.

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Because of the large number of recognized antibiotics resistance genes, parallel detection systems such as microarray are well suited to this task (Call et al., 2003).

Presently, PCR and hybridization analysis are common methods used to detect antibiotic resistance genes in bacteria. However, the detection of specific resistance genes remains a tremendous amount of work if every possible resistance gene has to be assessed, and therefore microarray technology is most suitable for resistance gene analysis (Holzman, 2003). A few microarrays have been developed for identifying antibiotics resistance genes (Call et al., 2003; Frye et al., 2006; Moneeke et al., 2003). This study describes a microarray technique for detecting the genes that confer resistance to aminoglycosides, beta-lactam, chloramphenicol, sulfonamide and tetracycline.

MATERIALS AND METHODS

Specimen collection, cultivation and identification of *Escherichia coli*

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. One gram of each animal's feces was homogenized in 9 ml of sterile saline solution, then the volume of the homogenate was made up to 10 ml to get a 10% suspension. The contents were mixed thoroughly and 10-fold serially diluted and 0.2 ml inoculums from each dilution plated out on Eosin Methylene Blue agar (EMB) (Oxoid, England). No antibiotic was included in the EMB agar plates used for the cultivation. The inoculated plates were incubated overnight at 37°C. A single colony on EMB with green metallic sheen taken to be *E. coli* was selected from an individual fecal sample for further characterization. *E. coli* was fully identified using conventional microbiological tests-Indole positive, methyl red positive and citrate negative (Cheesbrough, 2000). The cattle and goat specimens came from the herd at Obinze Owerri, Imo State while the Madonna University Poultry Okija, Anambra State was the source of poultry specimens. The specimens from swine came from a farm located at the Ogborhil area of Aba, Abia state.

Antibiotics susceptibility testing

The antibiotics susceptibility pattern of the isolates was determined using the disk diffusion method (Cheesbrough, 2000), on Mueller-Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2006). Susceptibility was tested against ampicillin (10 µg), amoxycillin/clavulanic acid (20/10 µg), tetracycline (30 µg), gentamicin (10 µg), cefpodoxime (10 µg), cefoxitin (30 µg), cefpirome (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), sulfamethoxazole-trimethoprim (10 µg), cephalothin (30 µg), nitrofurantoin, ceftriaxone (30 µg), and cefotaxine (30 µg) (Oxoid, England). *Escherichia coli* ATCC 25922 was included as a reference strain.

Preparation of microarray slides

Multiple DNA microarrays were printed on glass slides so that independent arrays were contained within ten individual wells defined by Teflon masking slides (Erie Scientific, Portsmouth, N.H. USA); the hydrophobic nature of the masking permitted independent samples to be hybridized within each well. Slides were derivatized with epoxysilane (3-glycidoxypropyltrimethoxysilane; (Sigma-Aldrich, Milwaukee, WS, USA) as described by Call et al. (2001). Prior to printing, the slides were soaked in 2.5% Contrad 70 detergent (Fisher Scientific, Pittsburgh, PA, USA.) for 2 min, rinsed three times with distilled water, and dried using compressed air. Slides were then soaked for 1 h in 3 N HCl, rinsed three times with deionized water, and dried with compressed air.

Construction of DNA microarray

Oligonucleotide probes of known antibiotics resistance genes were reconstituted in TE buffer, diluted to 60 µm in print buffer (0.1 M Na_2HPO_4, 0.2 M NaCl, 0.01% sodium dodecyl sulfate) with a pH of 11 and transferred to 384-microwell plates for printing. Arbitrary biotinylated oligonucleotides (70-mer; 5 µM) were included with every array. These biotin pseudoprobes served as positive controls for the detection chemistry and to orient the array for image processing. All probes were deposited as four replicates at a fixed location within each masked well using a Robotic Microgrid II arrayer (Bio-Robotics, Woburn, Mass.USA) with humidity held at 45%. Printing parameters included washing the pins in a recirculating bath (four pins washed twice for 4 s each time), followed by 0.5 s of flushing and 6 s of drying. This washing procedure was repeated twice between probes to minimize possible probe carry-over. Printed slides were baked under vacuum (22 Hg/mm) for 1 h (130°C) and stored away from light at room temperature until used.

Genomic DNA extraction

The bacterial total DNA was extracted using the Qiagen DNeasy silica-gel adsorption method (Qiagen, Valencia, CA USA).

A 1.0-ml volume of overnight broth culture of the test isolate was pelleted in a 1.5 ml microcentrifuge at 10000 rpm for 10 min and resuspended in180 µl of buffer ATL from the Qiagen DNeasy kit. Then 20 µl of Qiagen proteinase K solution was added, mixed by vortexing and the cell was incubated for 3 h in a 55°C shaker water bath for lysis. After the lysis, 20 µl of RNase A (100mg/mL) (Qiagen, Valencia, CA USA) was added to each tube (to degrade RNA) and the tubes were incubated at room temperature for two minutes. This was followed by the addition 200 µl of buffer AL, vortexing, and incubation at 70°C for 10 minutes. Then, the genomic DNA (gDNA) was concentrated by the addition of 200 µl of 100% ethanol. To separate the DNA from other cellular contaminants, the treated DNA lysate was pipetted into a DNeasy column in a collection tube, and centrifuged for 1 min at 10,000 xg. The remaining contaminants were washed out by using 500 µl each of buffer AW1 and AW2 in a new collection tube at each time. The purified gDNA was eluted in a fresh1.5 ml micro-centrifuge tube by using 200 µl AE buffer and centrifugation for 1 min at 10,000 xg. Finally, the nanodrop spectrophotometer was used to quantify the DNA. DNA was quantified to properly scale the subsequent nick translation and any sample that failed to reach the value of A260/A280 ratio of 1.7 to 2 or below 25 ng/µl was re-extracted. All the buffers, enzymes and columns used in this extraction came from the Qiagen DNeasy kit (Qiagen, Valencia, CA USA; Cat. No. 69504).

Nick translation: Biotinylation and fragmentation of DNA

This reaction is designed to generate small (50-100 base) biotin-labeled DNA probes by nick translation which are important for successful *in situ* hybridization.

Approximately 1.0 µg (up to 40ul) of the quantified gDNA, 5 µl of 10X dNTP mix [(0.2 mM each of dCTP, dGTP, dTTP; 0.1mM of dATP;

0.1mM of biotin-14-dATP; 500mM of Tris-HCl, pH 7.8; 100mM of β-mercaptoethanol and 100 µg/ml of nuclease-free BSA) (Invitrogen, USA)] and 5 µl of 10X enzyme mix [0.5U/µl of DNA polymerase 1, 0.007 U/µl of DNase 1, 50 mM of Tris-HCl pH 7.5, 5 mM of magnesium chloride, 0.1 mM of phenylmethylsulfonyl fluoride, 5% (v/v) of glycerol and 100 µg/ml of nuclease-free BSA) (Invitrogen, USA)] were combined in 0.2 ml PCR tubes on ice. The total volume was brought to 50 µl with PCR water. The mixture was incubated at 16°C in a thermal cycler for 2 h and then held at 4°C for nick translation of DNA. To precipitate the nick translated DNA, the samples were transferred to 1.5 ml micro-centrifuge tubes followed by the addition of 5 µl of 3 M sodium acetate, (pH 5.2), 110 µl of 100% ethanol and incubation at -80°C for 30 min. After the incubation, the DNA was pelleted by centrifugation at 14000 rpm for 30 min at 4°C. Then, the pellets were resuspended with 400 µl of 70% ethanol. For more purification, the above steps were repeated once and the pellets were dried with a vacuum centrifuge for 10 min. Finally, the purified nick-translated DNA was resuspended with 100 µl 1x hybridization buffer.

Microarray slide pre-hybridization preparation

Microarray slides were prepared by immersing them in 50 ml of 1% BSA blocking solution in a Coplin staining jar followed by incubation at room temperature for 10 min, with shaking at 80 rpm to eliminate bubbles on the slide surface. The slides were rinsed 20 times in double de-ionized after which their back and edges were wiped with a Kimwipe and spin dried with slide centrifuge for 15 s.

Sample application/hybridization

The nick translated gDNA was boiled for 3 min, chilled on ice and briefly vortexed for 15 s. Then, the microarray slides were placed on a humidified chamber (200 µl tip box and lid with de-ionized water covering the bottom of the box) and 45 µl of the gDNA sample was placed in each well (2 wells per nick translated gDNA sample) on the microarray slide. The droplets were carefully spread to fully cover the well without touching the slide surface with the pipette. Carefully, the slide was sealed (face-up and frosted end toward the cap) in a hybridization chamber (50 ml conical tube with filter paper moistened with 1x hybridization buffer). The slide was placed on top of the filter paper in the hybridization chamber without allowing the damp filter paper to touch the wells. The hybridization chamber was placed in a rack and lead weight on top of the rack, then the rack was submerged in the 55°C water bath. Finally, the sample DNA was allowed to hybridize with the probes on the array for 16 h.

Post-hybridization stringency washes

After hybridization, the slides were removed from the hybridization chamber with forceps and excess hybridization solution was aspirated off the slides. Then, the slides were completely immersed (frosted end up) in a 55°C pre-warmed low stringency array wash solution (1X SSC, 0.2% SDS) contained in a Coplin jar. The above procedure was repeated in medium stringency (0.1XSSC, 0.2% SDS) and high stringency (0.1XSSC) array wash solutions, respectively. At each time, the slides were washed for 4 min at room temperature on an Orbital shaker at 80 rpm. After the stringency washes, the slides were transferred to a horizontal staining jar that contains enough TNT buffer to cover the slide and were shaken for 1 min at 80 rpm at room temperature to remove the stringency wash buffers. This TNT buffer washing was repeated three times.

Microarray development

For the following applications, 45 µl of each solution was added directly to each well. The slides were gently tapped to distribute the reagent over the full well surface without allowing the reagents to cross over to other wells. The slides were spin-dried for 5 s using a slide centrifuge followed by incubation with 1:100 Streptvadin-Horseredish peroxidase (SA-HRP) in TNB for 30 min. After the incubation, the slides were washed 3 times for 1 min each in horizontal staining jars at 80 rpm shaking. The above procedure was repeated with 10% FES, 2XSSC; 1:50 BioT, 1xAmp Dil; and 1:500 SA-Alexa 555, 1XSSC, 5X Den. This last incubation was done for one hour in the dark. All incubation was done at room temperature in a humidified chamber (made from a covered tip box with ~10 ml PCR water in the bottom). At the end of these development reactions, the slides were spin-dried for 15 s using the slide centrifuge and were stored in the dark prior to scanning.

Scanning/imaging of slides

After hybridization and development, slides were scanned or imaged by standard DNA microarray slide scanners. The florescence marker used in this experiment (Alexa555) has an optimal excitation wavelength of 555 nm and emission wavelength of 565 nm. The scanner/imager we used (Applied Precision arrayWoRx scanner) has a white light source and an emission filter for Cy3 that functions well for Alex555. We used an excitation wavelength of 540 nm (25 nm bandwidth) and an emission wavelength of 595 nm (50 nm bandwidth).

There were five pairs of Teflon-masked wells on each slide, with each well containing a full array and our normal protocol calls for two wells to be hybridized to the same sample. Within each well there were two spots per probe so in effect there are four individual probe-target hybridizations (2 wells total). Each full array has dimensions of 22 horizontal and 20 vertical spots. The distance between spots is approximately 250 µm. Table 1 shows the oligonucluotide probes sequences used in constructing the DNA microarray.

RESULTS

Antimicrobial resistance genes for microarray construction

Ninety antimicrobial resistance genes oligonucluotide probes were employed in the microarray, they include 21 aminoglycoside resistance genes, *aac(3)-Id, aac(3)-III, aac(3)-Iva, aac(6')-Ib, aac(6')-IIa, aacC2, aacCA5, aadA1, aadA2, aadA21, aadA5, aadA7, aadB, aadE, aph(3)-Ia, aph(3)-IIa, aphA7, aphD, AphE, strA* and *strB;* 21 beta-lactam resistance genes, *blaACC-01, bla-CMY-2, blaCTX-M-1, blaCTX-M-12, blaCTX-M-15, blaCTX-M-2, blaCTX-M-8, blaDHA-1, blaFOX-2, blaIMP-2, blaKPC-3, blaMIR, blaOXA-1, blaOXA-2, blaOXA-7, blaOXY-K1, blaPSE-1, blaPSE-4, blaROB-1, blaSHV-37,* and *TEM1;* 10 chloramphenicol resistance genes, *cat4, catB2, catB3, catB8, catI, catII, catP, cmlA, cmlB,* and *floR;* 2 integrase genes, *intI1,* and *intI2,* 4 qinolone resistance genes, *qac delta E, qnrA1, qnrB* and *qnrS;* 11 trimethoprim resistance genes, *dfrA1, dfrA14, dfrA16, dfrA21, dhfrII, dhfrV, dhfrVI, dhfrVII, dhfrXII, dhfrXIII,* and *dhfrXV;* 3 sulfonamide resistance genes, *Sul1, Sul2,* and *sul3;* and 18 tetracycline resistance

Table 1. The oligonucluotide probes used in constructing the DNA microarray (Call et al., 2001, 2003).

Gene	Sequence	Description
aac(3)-Ia	CGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAACTTGCTCCGTAG	Aminoglycoside resistance
aac(3)-Ib	AAACAAAGTTAGGTGGCTCAATGAGCATCATTGCAACCGTCAAGATCGGCCCTGACGAAA	Aminoglycoside resistance
aac(3)-Id	TCAAGGCTATAGGCGCAGCGCGTGGAGCTTATGTGATTTACGTCCAAGCTGATAAAGGCG	Aminoglycoside resistance
aac(3)-III	CGACTGGCACTGTGATGGGATACGCGTCGTGGGACCGATCACCCTACGAGGAGACTCTGA	Aminoglycoside resistance
aac(3)-IVa	ACCATTCTTCAGGATGGCAAGTTGGTACGCGTCGATTATCTCGAGAATGACCACTGCTGT	Aminoglycoside resistance
aac(3)-Vb	ACCCTTCGATCTGGCCACATCCGGTACCTATCCCGGCTTCGGCCTGCTCAACCGGTTTCT	Aminoglycoside resistance
aac(6')-I30	TGGCCTGATATGAAAAGTGCCACCAAAGAAGTTGAAGAATGTATTGAGAAGCCAAACATA	Aminoglycoside resistance
aac(6')-Ib	CAATACACAGCATCGTGACCAACAGCAACGATTCCGTCACACTGCGCCTCATGACTGAGC	Aminoglycoside resistance
aac(6')-IIa	TGCTCCATGATTGGCTCAACCGGCCGCACATCGTTGAGTGGTGGGGTGGTGACGAAGAGC	Aminoglycoside resistance
aac(6')-Ia	TGGCCAGATATGACGAGTGCAACAAAAGAAGTAAAAGAATGTATTGAGAGTCCAAACCTT	Aminoglycoside resistance
aacC1	CCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCGTGAGTTCGGAGAC	Aminoglycoside resistance
aacC2	CGACTGGCACTGTGATGGGATACGCGTCGTGGGACCGATCACCCTACGAGGAGACTCTGA	Aminoglycoside resistance
aacCA5	TTGCGTTGGCTGCGGTTGACGAGCAAAAAGTCATTGGCGCTATCGCCGCGTATGAGTTGC	Aminoglycoside resistance
aadA1	GGCCTGAAGCCACACAGTGATATTGATTTGCTGGTTACGGTGACCGTAAGGCTTGATGAA	Aminoglycoside resistance
aadA2	GTTCCTGAACAGGATCTATTCGAGGCGCTGAGGGAAACCTTGAAGCTATGGAACTCGCAG	Aminoglycoside resistance
aadA21	GAGCGCCATCTGGAATCAACGTTGCTGGCCGTGCATTTGTACGGCTCCGCAGTGGATGGC	Aminoglycoside resistance
aadA5	CGGTGATCGAGCGCCATCTGGCTGCGACACTGGACACAATCCACCTGTTCGGATCTGCGA	Aminoglycoside resistance
aadA7	GGATCTCTTCAGCTCAGTCCCAGAAAGCGATCTATTCAAGGCACTGGCCGATACTCTGAA	Aminoglycoside resistance
aadB	TACTTTTACTATGCCGATGAAGTACCACCAGTGGACTGGCCTACAAAGCACATAGAGTCC	Aminoglycoside resistance
aadE	GAAGCATTATTTCTATGCCATCAATTGTTCAGGGCGGTATCCGGTGAGGTGGCGGAAAGG	Aminoglycoside resistance
aafA	CGTTGACAGGAGCGCAAATATCGACCTGAGTTTTACTATTAGACAACCGCAACGCTGCGC	*E. coli* pathotype
aap	GGGACGGGTCCACATTATCTGCGTTCCAACCGCTACCACCCGCAAAGGCATTCAGGCTGA	*E. coli* pathotype
aatA	ACAGGGAGGTGCATTGGGTAATATGAGTCTCAGAAAAATGGATTATAGTGCTAGTCTGGG	*E. coli* pathotype
abe (C2-C3)	TGTCCTATTACCAACAAGACTGCTTGAGTTAATGCCAGCGCTTAAAACGAAATTCTTTAT	Serogrouping
aggA	CGACGACAGAGCAATGTGCTAAAAGCGGTGCAAGGGTCTGGTTATGGGGAACAGGTGCCG	E coli pathotype
aidaI	GGCCTACAGTATCATATGGAGCCACTCCAGACAGGCCTGGATTGTGGCCTCAGAGTTAGC	Virulence
aph(3)-Ia	ATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGC	Aminoglycoside resistance

Table 1. Contd.

aph(3)-IIa	TAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCA	Aminoglycoside resistance
aph4	GGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATGGTTTCTACAAAGATCGTTATG	Aminoglycoside resistance
aphA-3	TCTTTCACTCCATCGACATATCGGATTGTCCCTATACGAATAGCTTAGACAGCCGCTTAG	Aminoglycoside resistance
aphA7	CCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTAC	Aminoglycoside resistance
aphD	CTGCAGAACACCCTGTGGGACATCGAGGACGGGCTGACGGCGATCGCCCCCTCCCAGATC	Aminoglycoside resistance
AphE	GTCGTCTGCCACGGTGATCTCTGCCTGCCCAACATCGTCCTCCATCCGGAGACCCTGGAG	Aminoglycoside resistance
aphIII	CTCCTGCTAAGGTATATAAGCTGGTGGGAGAAAATGAAAACCTATATTTAAAAATGACGG	Aminoglycoside resistance
bfpA	GGTGCTTGCGCTTGCTGCCACCGTTACCGCAGGTGTGATGTTTTACTACCAGTCTGCGTC	E coli pathotype
bla carb-2	GCGTTACGCCGTGGGTCGATGTTTGATGTTATGGAGCAGCAACGATGTTACGCAGCAGGGG	Beta-lactam resistance
blaACC-01	CAGCCGCTGATGCAGAAGAATAATATTCCCGGTATGTCGGTCGCAGTGACCGTCAACGGT	Beta-lactam resistance
bla-CMY-2	TTATGCTGCGCTCTGCTGCTGACAGCCTCTTTCTCCACATTTGCTGCCGCAAAAACAGAA	Beta-lactam resistance
blaCTX-M-1	GCGGCACACTTCCTAACAACAGCGTGACGGTTGCCGTCGCCATCAGCGTGAACTGACGCA	Beta-lactam resistance
blaCTX-M-12	GGGTGTGGCATTGATTAACACAGCGGATAATTCGCAAATACTTTATCGTGCTGATGAGCG	Beta-lactam resistance
blaCTX-M-14	CGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTC	Beta-lactamase CTX-M-14
blaCTX-M-2	GCAACGCTGCATGCGCAGGCGAACAGCGTGCAACAGCAGCTGGAAGCCCTGGAGAAAAGT	Beta-lactam resistance
blaCTX-M-8	TTTCGCTGTTGCTGGGGAGTGCGCCGCTGTATGCGCAGGCGAACGACGTTCAGCAAAAGC	Beta-lactam resistance
blaDHA-1	CGGATTCTATGACAGCCATCCGCATATTGATCTGCATATCTCCACCCATAACAATCATGT	Beta-lactam resistance
blaFOX-2	CAAGATGCAAACTTACTATCGGAGCTGGTCACCGGTTTATCCGGCGGGGACCCATCGCCA	Beta-lactam resistance
blaIMP-2	TTTGTGGAGCGCGGCTATAAAATCAAAGGCACTATTTCCTCACATTTCCATAGCGACAGC	Beta-lactam resistance
blaKPC-3	GTTACGGCAAAAATGCGCTGGTTCCGTGGTCACCCATCTCGGAAAAATATCTGACAACAG	Beta-lactam resistance
blaMIR	TCCGAAAAACAGCTGGCTGAGGTGGTGGAACGTACCGTTACGCCGCTGATGAACGCGCAG	Beta-lactam resistance
blaOXA-1	ACCTTCAGTTCCTTCAAATAATGGAGATGCGACAGTAGAGATATCTGTTGATGCACTGGC	Beta-lactam resistance
blaOXA-2	CCACAATCAAGACCAAGATTTGCGATCAGCAATGCGGAATTCTACTGTTTGGGTGTATGA	Beta-lactam resistance
blaOXA-27	GAAAAGGTCATTTACCGCTTGGGAAAAAGACATGACACTAGGAGAAGCCATGAAGCTTTC	Beta-lactam resistance
blaOXA-7	GCAGGCTAATTTACTGCTACTTTTACAAAGCACGAAAACACCATTGACGGCTTCGGCAGA	Beta-lactam resistance
blaOXA-9	GCTCGTCTTTTAAACTTCCATTGGCAATCATGGGGTTTGATAGTGGAATCTTGCAGTCGC	Beta-lactam resistance
blaOXY-2b	TAAAGAGGTGGTAAATAAAAGGCTGGAGATTAACGCAGCCGATTTGGTGGTCTGGAGCCC	Beta-lactam resistance
blaOXA-61	GGAAAAACTTGGGCGAGTAACGACTTTTCAAGGGCTATGGAGACTTTCTCTCCCGCTTCC	Beta-lactam resistance

Table 1. Contd.

blaOXY-K1	ACCAATGATATTGCGGTTATCTGGCCGGAAGATCACGCTCCGCTGATATTAGTCACCTAC	Beta-lactam resistance
blaPER-2	GAAATGGATGGTTGAAACCACCACAGGACCACAGCGGTTAAAAGGCTTGTTACCTGCTGG	Beta-lactam resistance
blaPSE-1	AGTGAGCATCAAGCCCCAATTATTGTGAGCATCTATCTAGCTCAAACACAGGCTTCAATG	Beta-lactam resistance
blaPSE-4	CGTTCAGTATTGCCGGCGGGATGGAACATTGCGGATCGCTCAGGTGCTGGCGGATTTGGT	beta lactam resistance
blaROB-1	TTGCTGACATTAACGGCTTGTTCGCCCAATTCTGTTCATTCGGTAACGTCTAATCCGCAG	Beta-lactam resistance
blaSHV-37	GCAAATTAAACTAAGCGAAAGCCAGCTGTCGGGCCGCGTAGGCATGATAGAAATGGATCT	Beta-lactam resistance
Cat	CGACATGAAGAGTTCAGGACCGCATTAGATGAAAACGGACAGGTAGGCGTTTTTTCAGAA	Phenicol resistance
cat4	CCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGAAATTCCGTATGGCAATGA	Phenicol resistance
catB2	TCGGCAGCTTCTGCTCCATCGGATCAGGCGCAGCTTTTATTATGGCTGGGAATCAAGGCC	Phenicol resistance
catB3	GGGCGGTACAGCTATTACTCTGGCTACTATCATGGGCACTCATTCGATGACTGCGCACGG	Phenicol resistance
catB8	GCTTTTGTTCTATAGGAAGCGGGGCTTCCTTCATCATGGCTGGCAATCAGGGGCATCGGC	Phenicol resistance
catI	GGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAAC	Phenicol resistance
catII	TAATATCGAGTTTGGTGGTCAGGCTGAATCCGCATTTAATCTGCTGACGATAAAGGGCAA	Phenicol resistance
catII	TTGTTAAGCTAAAACCACATGGTAAACGATGCCGATAAAACTCAAAATGCTCACGGCGAA	Phenicol resistance
catP	TGGCAATTCAAGTTCATCACGCAGTATGTGACGGATTTCACATTTGCCGTTTTGTAAACG	Phenicol resistance
cblA	AAACATATCAATGACTATATCCACCGGTTGAGTATCGACTCCTTCAACCTCTCGGAAACA	Beta-lactam resistance
Cif	TGAAAGACATTACCCTTCCCCCCCCGACGTCCGCGTCCTGTCTGACAGGGGCCATATCTG	Virulence
cmlA	GGCATCACTCGGCATGGACATGTACTTGCCAGCAGTGCCGTTTATGCCAAACGCGCTTGG	Phenicol resistance
cmlB	TCATCTACGGCTTGCTTGGCTCTATGCTTGCTATGGTTCCGGCGATAGGCCCATTGCTGG	Phenicol resistance
Dfr1	AGCCGGAAGGTGATGTTTACTTTCCTGAAATCCCCAGCAATTTTAGGCCAGTTTTTACCC	Trimethoprim resistance
dfrA1	GCGGTCGTAACACGTTCAAGTTTTACATCTGACAATGAGAACGTAGTGATCTTTCCATCA	Trimethoprim resistance
dfrA14	ACCTACAATCAGTGGCTTCTGGTGGGTCGCAAGACGTTTGAATCTATGGGCGCACTCCCC	Trimethoprim resistance
dfrA16	ATGGCTGCCAAGTCGAAGAACGGTATTATCGGTAATGGACCAGATATTCCATGGAGCGCC	Trimethoprim resistance
dfrA19	GCAGTTAGAAAAGGATGGCGCCGAGGAGCGAATCAAGGAGAAAGGAATTCTCCCCGAACG	Trimethoprim resistance
dfrA21	GGTCGTTATGGGCCGCAAGACATTTGAGTCCATAGGCAAGCCCTTACCAAACCGCCACCAC	Trimethoprim resistance
dfrA23	TGGCTTGTGCATTACCGTCATGTGGACTTTTGTGGCAGATGCGAGGGCTTGCACGTACAG	Trimethoprim resistance
dhfrI	GGTTAAAGCATCTTTAATTGATGGAAAGATCAATACGTTCTCATTGTCAGATGTAAAACT	Trimethoprim resistance
dhfrII	GCACAAAACTCACTCCTGAAGGCTATGCGGTCGAGTCCGAATCCCACCCAGGCTCAGTGC	Trimethoprim resistance

Table 1. Contd.

dhfrIII	ACTTGATTGGCAAAGATAATCTTATTCCATGGCATCTACCTGCCGATCTGCGTCATTTCA	Trimethoprim resistance
dhfrIX	AAACAAAACTTATTTTCCAAATTTGGATTAACCCTAACCCTATTAGTGAGGAACCCACAT	Trimethoprim resistance
dhfrV	TTCCGAATATTCCCAATACCTTCGAAGTTGTTTTTGAGCAACACTTTAGCTCAAACATTA	Trimethoprim resistance
dhfrVI	TCTTTGTTTCTGGTGGTGGTGAAATATATAAAGCTTTAATCGATCAAGCAGATGTTATCC	Trimethoprim resistance
dhfrVII	GAACACCCATAGAGTCAAATGTTTTCCTTCCAACAAGGAGCCACTGATTATATGTGAGCG	Trimethoprim resistance
dhfrVII	AATGGCATGGAAGAACATGACCTTCACACTTACTTCACTTACCGTAAAAAGGAGCTTACA	Trimethoprim resistance
dhfrX	ATGTGTATGTACCGGTAGAACTAATGAATAAACTCTATAGTGATTTCAAATATCCAGAAA	Trimethoprim resistance
dhfrXII	ATTGGCAATGGTCCTAATATCCCCTGGAAAATTCCGGGTGAGCAGAAGATTTTTCGCAGA	Trimethoprim resistance
dhfrXIII	AGTGCTTAACGCAGCAGAATTCGAGGTTGTCTCATCCGAAACCATTCAAGGCACAATCAC	Trimethoprim resistance
dhfrXV	CGATAAAGTTGATACTTTACATATTTCAACAATCGACATTGAGCCAGAAGGTGATGTCTA	Trimethoprim resistance
DT104	CTAATGCGTTTGGTCTCACAGCCGATGCGGTGCTGGCGGAATATCGTCACTGGCGTAACG	DT104 marker
Eae	TTATGCGGCACAACAGGCGGCGAGTCTCGGTAGCCAGCTTCAGTCGCGATCTCTGAACGG	*E. coli* pathotype
Eaf	CGTGCAGGTCGCCTGTTCGAAACGCTGGCTCAGGGACGGGTGGATGGTAGCTGGCTTAAT	*E. coli* pathotype
ehxA	TACCAGACCTGGGCCCCCTGGGGGATGGGCTGGATGTTGTCTCCGGAATTCTTTCTGCTG	*E. coli* pathotype
Ent	TTAATCGCGCCGCCATGCTGTTCGATGATATTTTGCACCACAGCCAGCCCCAGGCCTGTC	Virulence
espC	TGGCAGCTTTGTCAACAGCAGCCTGACCCTCGAAAAAGGAGCAAAACTAACGGCTCAGGG	E coli pathotype
estA	AGCTAATGTTGGCAATTTTTATTTCTGTATTATCTTTCCCCTCTTTTAGTCAGTCAACTG	Virulence
f165(1)A	CTGGGCCACAAGTAACGGGGCAGGCTGAAGAATTAGCAACTAACGGCGGTACGGGCACAG	*E. coli* pathotype
fliC	ATGAAGTTTCCGTTGATAAGACGAACGGTGAGGTGACTCTTGCTGGCGGTGCGACTTCCC	Virulence
fliCH7	CCCGCGGTAAACCCAATAGTTTTGCTCAGTACACCGGAATTAAAGGTAATTGAAGATGTC	Virulence
floR	GCGTGGGATGGCGTTGCTTGTTTGCGGAGCGGTCCTGTTGGGGATCGGCGAACTTTACGG	Phenicol resistance
fotA	CCTCTGCGCGCATACATTGGTACCTTAAATGGCCAGCCAGGTGTTTTGGGCAATGCGGCC	*E. coli* pathotype
hlyA	TAGTGCTGCTGCAACGACATCTCTGGTTGGTGCACCGGTAAGCGCGCTGGTAGGGGCTGT	*E. coli* pathotype
IncFII / Ori	TAGCGCTAACCGATGGTTTTGCAAAGCGCTAACCGTCAGTCTTTCAGGGTGCGTGGTTCC	Replicon typing
IncN / kikA	CTTCAATATCGTTAAAAAGAACAAGCACGGCTTTTTACCCAACCACACGAAGGATGCTAG	Replicon typing
IncP / trfA2	ACGGATGTTCGACTATTTCAGCTCGCACCGGGAGCCGTACCCGCTCAAGCTGGAAACCTT	Replicon typing
IncW / trwAB	AGCGTATGAAGCCCGTGAAGGGCGAATTGAAGCGCCTTGGCATTGAGGTTTGGACACCGG	Replicon typing
intI1	CTACTTGCATTACAGTTTACGAACCGAACAGGCTTATGTCAACTGGGTTCGTGCCTTCAT	Integrase gene

Table 1. Contd.

intI2	ATGAATGCTTGCGTTTGCGGGTTAAAGATTTTGATTTTGATAATGGCTGCATCACTGTGC	Integrase gene
invA	GTACCAGCCGTCTTATCTTGATTGAAGCCGATGCCGGTGAAATTATCGCCACGTTCGGGC	Virulence
invX	CAGACAGTGACTCAACTTCAAGAGCAGACACTTCCTTTTGGTATAAAGCTTATAGGTGTC	*E. coli* pathotype
ipaB	GGGGGCAATCGCAGGCGCTCTTGTCTTGGTTGCAGCAGTCGTTCTCGTAGCCACTGTTGG	*E. coli* pathotype
Iterons	CGCGAATCGTCCAGTCAAACGACCTCACTGAGGCGGCATATAGTCTCTCCCGGGATCAAA	Replicon typing
Iterons	AACGGGACGACTATGACAACGGTAGTGACTTGCTGGGCTCACTACCATTGTCACCCTGTG	Replicon typing
Iterons	CGGCGTTGTGGATACCTCGCGGAAAACTTGGCCCTCACTGACAGATGAGGGGCGGACGTT	Replicon typing
leoA	TGTCCTGCGTATTGCTCTGTTGGGGGCGTTCTCCGATGGCAAAACCAGCGTTATCGCCGC	*E. coli* pathotype
Lt	TTTTATGTTTTATTTACGGCGTTACTATCCTCTCTATGTGCACACGGAGCTCCTCAGTCT	Virulence
LTIIa	GTGTGCCGAATAATAAAGAATTTAAAGGAGGGGTGTGCATTTCAGCGACAAATGTGCTAT	Virulence
Mpha	ACCCACCGACGTCCATCGTCGACGGTGGCGATCACGATCCTATAGTCGAGCCCAAGCTCA	macrolide 2'-phosphotransferase
ori γ	GCTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGC	Replicon typing
OtrB	GATCAACCTTGACGACACGTCCCTGCTGAACGGCATCGACGCCCGGCTGATGCAGCCGGT	Tetracycline resistance
pagC	TGGTTGGGCCAGCCTATCGATTGTCTGACAATTTTTCGTTATACGCGCTGGCGGGTGTCG	Virulence
papGI2	GCTCAGGTCCAGATGTTGCGAGCGGCGTATATTTCCAAGAGTACCTGGCCTGGATGGCAG	*E. coli* pathotype
parA-parB	TGCTGGTAGACCGCCATCACGGATTCTTCGGCAACATCAAGCTGTTTGGGAGAGCAGAGC	Replicon typing
Pet	ATCTATGTCGCCGGTGGCCCGGGCACAGTACAACTCAATGCAGAGAACGCCCTGGGTGAG	*E. coli* pathotype
Pir	AATTCGCCACCGAAACGAGCTAAATCACACCCTGGCTCAACTTCCTTTGCCCGCAAAGCG	Replicon typing
qac delta E	GCAGTCTGGTCGGGACTCGGCGTCGTCATAATTACAGCCATTGCCTGGTTGCTTCATGGG	Disinfectant resistance
qnrA1	CAGCAAGAGGATTTCTCACGCCAGGATTTGAGTGACAGCCGTTTTCGCCGCTGCCGCTTT	Qinolone resistance
qnrB	AACTCCGAATTGGTCAGATCGCAATGTGTGAAGTTTGCTGCTCGCCAGTCGAAAGTCGAA	Qinolone resistance
qnrS	CGTGCTAACTTGCGTGATACGACATTCGTCAACTGCAAGTTCATTGAACAGGGTGATATC	Qinolone resistance
repA FIB	ACACCGTACAACCTGTGGCGCTGATGCGTCTGGGCGTTTTTGTACCGACCCTTAAATCAC	Replicon typing
repA FIC	CATTTGGGACCAAAAGCGTGAGCACGAAGACCTGTCCAACGCCGTAGTGACGCGACAATG	Replicon typing
repA FIIS	CTGATGGCGAAAGCCGAAGGGTTCACGTCCCGTTTTGATTTTTCCGTCCATGTGGCGTTC	Replicon typing
repA L/M	ACCTACAGCTTTCTGACATTGAGTCAGTAGAAGGTCTTTCGCCGGAGTTCATCTCCTGGC	Replicon typing
repA N	AGCCGTTCTGCGGTAATCTTTTACCCGAAAGAAGGGAGTTTTGACTGCGTCGCGCGCCCC	Replicon typing
repA T	AAGCCCTTCCACGTCTAGAAGTTGCACAAGCCCTGTATACCTTCCTTGCAAGCCTTCCAA	Replicon typing

Table 1. Contd.

repA W	AACAAAGCCCCCGGCCATCGTATCAACGAGATCATCAAGACGAGCCTCGCGCTCGAAATG	Replicon typing
repA Y	ACACTGTGCAGCCTGTAGCGTTGATGCGCTTGGGGGTATTCGTGCCGAAGCCATCAAAGA	Replicon typing
repA2 FIC	GATGAGGAAGGTATTACCCAGGCGCAGATGCTTGAAAAACTGATTGAATCAGAGCTGAAA	Replicon typing
repAB L/M	ATGCGTACCCTATTGCAATACAGCCCGGCCAATATGTGCAGGGGCTGGTGAATCAAAAGA	Replicon typing
repC L/M	GTAGTTGAGCGGCAGGTGCATAAGAGTAACCTGGATAAGCAGAAGGATTACAGGAATCGC	Replicon typing
rfbE	ATGTCTGTTAGTGACATAGAACAAAAAATCACTAATAAAACTAAAGCTATTATGTGTGTC	Virulence
rfbE (A_D)	CCTACCCAGCCTTGATCATAAGTAGCAAACTGTCTCCCACCATACATTGATGAATGCCTG	Serogrouping
RNAI	AACGGCAGAATGCGCCATAAGGCATTCAGGACGTATGGCAGAAACGACGGCAGTTTGCCG	Replicon typing
RNAI	CAGGAGAGATGGCATGTACGGGCAGTAAGTCAGAAGACTGAAGATGTTCCGGAAGCCATA	Replicon typing
RNAI	AGAATGCGCCATAAGGCATTCAGGATGTATGGCAGAAACGACGGCAGTTTGCCGGGGCCG	Replicon typing
RNAI/repA	TGGCTGGCCACGCCGTAAGGTGGCAAGGAACTGGTTCTGATGTGGATTTACAGGAGCCAG	Replicon typing
Saa	CTTGGTAGCGGTAAAACGGAGGCAGGGGGAAGAGCATCTGCTACAGGAGTTGATTCGACC	E coli pathotype
sefA	GGGAGCCAATATTAATGACCAAGCAAATACTGGAATTGACGGGCTTGCAGGTTGGCGAGT	Salmonella-specific
sfaA	GCCCTGACCTTGGGTGTTGCGACAAATGCGTCTGCTGTCACCACGGTTAATGGTGGTACA	*E. coli* pathotype
sfaD	TCCCGCTGCACTGGCCGGAAACCACTGGCATGTCATGCTTCCGGGAGGAAACATGCGCTT	*E. coli* pathotype
sfaHII	GACCTTCCGTCCTATCCCGGAGGGCCGGTAACAGTCCCTCTTACTGTACGTTGCGACCAG	*E. coli* pathotype
sipA	CTCAGCCCCCCGTCATAATGCCAGGTATGCAGACCGAGATCAAAACGCAGGCCACGAATC	Virulence
sipB	GTGGCAACGAAAGCGGGCGACCTTAAAGCCGGAACAAAGTCCGGCGAGAGCGCTATTAAT	Virulence
sipC	AGCGCTAAAGATATTCTGAATAGTATTGGTATTAGCAGCAGTAAAGTCAGTGACCTGGGG	Salmonella-specific
sopA	CCCCTCAGGTATGGACCGACCAGAGCTGGCATCCCAATACGCATCTCCGTGATGCTAACG	*E. coli* pathotype
spvC	GCGGAAGATGCCGGTATCCCACTTTAAAGAGGCGCTGGATGTGCCTGACTATTCAGGGAT	Virulence
spvR	CTGCCAGAAATTATTTTCATCGGGAATCGCTTGTCTGCCGGACATCAGTGGAGGGTGGGG	Virulence
SSpp	CGTCAAAAAGTGAAGGAAATTACGCTGCATTTATTATGGATCAGAATACGCCCCGTTCGG	Salmonella-specific
Stb	AGAATATCGCATTTCTTCTTGCATCTATGTTCGTTTTTTCTATTGCTACAAATGCCTATG	Virulence
stII	CGCATTTCTTCTTGCATCTATGTTCGTTTTTTCTATTGCTACAAATGCCTATGCATCTAC	*E. coli* pathotype
strA	ACGCGCCGTTGATGTGGTGTCCCGCAATGCCGTCAATCCCGACTTCTTACCGGACGAGGA	Aminoglycoside resistance
strB	GGTGCCTTTCCGCAGCTTGGAACGCGGATGGAGAAGAGGAGCAACGCGATCTAGCTATCG	Aminoglycoside resistance
stx1A	CTGGTGACAGTAGCTATACCACGTTACAGCGTGTTGCAGGGATCAGTCGTACGGGGATGC	*E. coli* pathotype
stx1B	CGCTTTCATTTTTTTCAGCAAGTGCGCTGGCGACGCCTGATTGTGTAACTGGAAAGGTGG	*E. coli* pathotype

Table 1. Contd.

Stx2A	CCATGACAACGGACAGCAGTTATACCACTCTGCAACGTGTCGCAGCGCTGGAACGTTCCG	*E. coli* pathotype
Stx2B	GCAATGGCGGCGGATTGTGCTAAAGGTAAAATTGAGTTTTCCAAGTATAATGAGGATGAC	*E. coli* pathotype
Sul1	CCCGCACCGGAAACATCGCTGCACGTGCTGTCGAACCTTCAAAAGCTGAAGTCGGCGTTG	Sulfonamide resistance
Sul2	GCGCTCAAGGCAGATGGCATTCCCGTCTCGCTCGACAGTTATCAACCCGCGACGCAAGCC	Sulfonamide resistance
sul3	GATTGATTTGGGAGCCGCTTCCAGTAATCCTGATACAACTGAAGTGGGCGTTGTGGAAGA	Sulfonamide resistance
TEM1	CCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAC	Beta-lactam resistance
tet(C)	GACTGGCGATGCTGTCGGAATGGACGATATCCCGCAAGAGGCCCGGCAGTACCGGCATAA	Tetracycline resistance
tet(Y)	GCGTTTATGCAGGTCTTTTGCGCGCCCGTTTTAGGGCGGTTATCTGACCGCTATGGACGG	Tetracycline resistance
Tet30	CGACCGGTTCGGTCGGCGCCCGGTCTTGTTGCTTTCTTTGGCCGGTACCCTGCTTGATTA	Tetracycline resistance
TetA	GCGGCTTCTATAACAACGTGGAACGGGTGGGCATGGATTGCAGGCGCTGCCCTCTACTTG	Tetracycline resistance
tetB	TGGATGCTGTATTTAGGCCGTTTGCTTTCAGGGATCACAGGAGCTACTGGGGCTGTCGCG	Tetracycline resistance
TetD	GGCTATCGGCGGACTGGCGGGGGATATCTCACCGCATCTGCCGTTTGTCATTGCGGCAAT	Tetracycline resistance
TetE	GTTGAGGCTGCAACAGCTCCAGTCGCACCGGTAATACCAGCAATTAAGCGTCCCAAATAC	Tetracycline resistance
tetG	ACGGGTTCGCGTTCCTGCTTGCCTGCATTTTCCTCAAGGAGACTCATCACAGCCATGGCG	Tetracycline resistance
TetH	GGCGCATCATTGCGGGGATCACAGGCGCAACAGGTGCCGTATGTGCATCAGCGATGAGTG	Tetracycline resistance
TetJ	CCCATGTTAGGGGGATTACTCGGTGAGATCAGCGCCCATACGCCATTTATCTTTGCGGCT	Tetracycline resistance
TetK	TTGGTAGGTTAGTACAAGGAGTAGGATCTGCTGCATTCCCTTCACTGATTATGGTGGTTG	Tetracycline resistance
TetM	GGATATTAAAGAGAAACTTTCTGCCGAAATTGTAATCAAACAGAAGGTAGAACTGTATCC	Tetracycline resistance
TetO	ACGGAACGTTATTTCCCGTTTATCACGGAAGCGCTAAAAACAATCTGGGGACTCGGCAGC	Tetracycline resistance
TetQ	GTGCCGCCCAACCCTTATTGGGCCACAATAGGGCTGACTCTTGAACCCTTACCGTTAGGG	Tetracycline resistance
TetS	CAGAAATGTATACTTCAATAAATGGAGAATTACGCCAGATAGATAAGGCAGAGCCTGGTG	Tetracycline resistance
TetT	GCTACAACGACAACGGATTCGATGGAACTTGAAAGAGATAGGGGAATAACTATACGGGCG	Tetracycline resistance
TetU	GCAGCTAAGACGTGGCAAAGCAACGGATTGGCATGCGATGGTTCAGGAAAGCTTAGATAG	Tetracycline resistance
TetV	CGTCGCGAAGATCACCTCCATCGAGACCACCTTCGACAGCGGACCCACGATCGCGAATGA	Tetracycline resistance
TetW	AACGATGTATTAGGGGACCAAACCCGGCTCCCTCGTAAAAGGTGGCGCGAGGACCCCCTC	Tetracycline resistance
TetX	CGACCGAGAGGCAAGAATTTTTGGTGGAACCCTTGACCTACACAAAGGTTCAGGTCAGGA	Tetracycline resistance

genes, *tet(C), tet(Y), Tet30, TetA, tetB, TetD, TetE, tetG, TetH, TetJ, TetK, TetM, TetQ, TetS, TetT, TetV, TetW* and *TetX.*

The sequence identity of each gene was compared with GenBank sequences, therefore, all the 90 genes were used to construct the DNA microarray; biotin was used as the positive control and 16s rRNA as orientation.

Few virulence and virulence related genes were also

Table 2. The Prevalence of aminoglycosides resistance genes in *E. coli* Isolates.

Genes	S O R C	E S O F	S A M	P L E	S
	Pig N=12	Goat N=10	Poultry N=8	Cattle N=10	Total N=40
aac(3)-Id	03(25)	00	05(62.5)	01(10.0)	08(20)
aac(3)-III	04(33.3)	00	03(37.5)	02(20)	09(22.5)
aac(3)-IVa	00	00	00	00	00
aac(6')-Ib	00	00	01(12.5)	00	01(2.5)
aac(6')-IIa	00	00	00	01(10)	01(2.5)
aacC2	00	00	00	00	00
aacCA5	02(16.7)	04(40)	02(25)	02(20)	10(25)
aadA1	01(8.3)	02(20)	01(12.5)	05(50)	09(22.5)
aadA2	01(8.3)	02(20)	01(12.5)	05(50)	09(22.5)
aadA21	01(8.3)	02(20)	01(12.5)	04(40)	08(20)
aadA5	01(8.3)	00	02(25)	01(10)	04(10)
aadA7	00	00	00	00	00
aadB	00	00	00	00	00
aadE	07(58.3)	07(70)	07(87.5)	07(70)	28(70)
aph(3)-Ia	02(16.7)	00	01(12.50	00	03(7.5)
aph(3)-IIa	04(33.3)	00	05(62.5)	02(20)	11(27.5)
aphA7	02(16.7)	01(10)	03(37.5)	00	06(15)
aphD	05(41.7)	03(30)	06(75)	02(20)	16(40)
AphE	06(50)	01(10)	04(50)	03(30)	14(35)
strA	08(66.7)	07(70)	07(87.5)	06(60)	28(70)
strB	03(25)	04(40)	03(37.5)	03(30)	1332.5)

N = Number of isolates hybridized.

included in the array for differentiating the isolates into various pathotypes. To determine the specificity of microarray hybridization, all of the labeled genes probes were hybridized to the microarray. In most cases there was a one-to-one correspondence for hybridization signal to respective target, orientation gene, and positive control gene spots. There was minor cross-hybridization between some genes and they were marked as abnormal during analysis, thus these genes are not included in the net results shown here.

Detection of antimicrobial resistance gene with microarray

Forty (40) *E. coli* isolates were tested for antimicrobial resistance genes with the microarray. Thirty seven isolates were identified as having at least one antimicrobial resistance gene. Three remaining isolates (CA2, cow; GO3, goat; PL18, poultry) did not hybridize to any of the resistance genes presented on the array. Multiple antimicrobial resistance genes belonging to same category of antimicrobials were detected in most isolates.

Among the aminoglycosides, the most prevalent resistance genes were *aadE* and *strA,* 28 (70%) respectively, the most prevalent host were the isolates from poultry 07 (87.5%) (Table 2). The most encountered beta-lactam gene in this study was *bla-CMY-2,* 28(70%). However, *blaCTX-M-12* and *blaIMP-2* were detected only in isolates from poultry specimens (Table 3). The most prevalent chloramphenicol resistance genes observed in this study was *floR,* 22 (55.0%), while Integrase gene, int1 had the highest occurrence rate of 37.5% (15 isolates) (Table 4). In the trimethoprim and sulfonamide resistance gene families, the most prevalent was *dhfrV,* which was detected in 9 isolates (22.5%). For sulfonamide resistance genes, 14 isolates (35%) of the animal specimens harbored *Sul2* at highest rate. The *dhfrII* gene was only detected in isolates from pigs and poultry (Table 5). Among the tetracycline resistance genes, *TetA* was most with 21 isolates (52.5%) of animal specimens bearing this gene (Table 6). A sample micrograph of microarrays hybridized with genomic DNAs of the *E. coli* isolates are shown in Figure 1.

DISCUSSION

DNA microarrays have been used previously to detect resistance genes in bacteria (Call et al., 2003; Frye et al., 2006; Moneeke et al., 2003; Van Hoek et al., 2005; Ma et

Table 3. The prevalence of beta-lactam resistance genes in *E. coli* isolates.

Gene	SOURCES				
	Pig N=12	Goat N=10	Cattle N=10	Poultry N=8	Total N=40
blaACC-01	01(8.3)	00	01(10)	01(12.5)	03(7.5)
bla-CMY-2	09(75)	06(60)	06(60)	07(87.5)	28(70)
blaCTX-M-1	00	00	01(10)	04(50)	05(12.5)
blaCTX-M-12	00	00	00	01(12.5)	01(2.5)
blaCTX-M-15	02(16.7)	01(10)	00	03(37.5)	06(15)
blaCTX-M-2	00	00	00	00	00
blaCTX-M-8	01(8.3)	02(20)	02(20)	04(50)	09(22.5)
blaDHA-1	00	00	00	02(25)	02(5.0)
blaFOX-2	00	00	00	00	00
blaIMP-2	00	00	00	01	01(2.5)
blaKPC-3	06(50)	03(30)	02(20)	06(75)	17(42.4)
blaMIR	02(16.7)	02(20)	01(10)	02(25)	07(17.5)
blaOXA-1	03(25)	00	00	04(50)	07(17.5)
blaOXA-2	00	00	00	00	00
blaOXA-7	00	00	00	00	00
blaOXY-K1	01(8.3)	00	00	00	01(2.5)
blaPSE-1	02(16.7)	01(10)	00	02(25)	05(12.5)
blaPSE-4	07(58.3)	07(70)	04(40)	07(87.5)	25(62.5)
blaROB-1	01(8.3)	00	00	00	01(2.5)
blaSHV-37	01(8.3)	02(20)	01(10)	02(25)	06(15.0)
TEM1	02(16.7)	01(10)	03(30)	05(62.5)	11(27.5)

N = Number of isolates hybridized.

al., 2007; Greg et al., 2010). Several types of DNA templates can be used to construct microarrays, depending on the intended use. For example, short oligonucleotide probes can be used to detect single nucleotide polymorphism, long oligonucleotide probes can be used to detect sequences that contain a few mismatches, and PCR probes can be used to detect moderately divergent genes. In the present study, oligonucleotide probes were used to construct microarrays that could identify up to ninety genes that confer resistance to variety of antibiotics used in combating Gram-ve bacteria like *E. coli.*

When compared with phenotypic testing, microarrays have the advantage of detecting the presence of antibiotic resistance genes that are not phenotypically expressed (Peterson et al., 2009). In this study, antibiotic resistance genes of 40 *E. coli* isolates from variety of domestic live stock viz cattle, goats, swine and poultry in south eastern states of Nigeria were detected. It was observed that microarray detected genes that were not phenotypically expressed in the following isolates, PG6, PG 11-Swine *(aadE, floR, OtrB, qnrA1, strA, TetD, strA)*; CA 12-Cattle (*Aph E)* and PL 7-Poultry (*aadE, aphA7, bla-CMY-2, blaOXA-1, blaPSE-4, floR, IncFII/OriB, IncP / trfA2, qnrA1, strA, TetE, TetJ).* Ma et al. (2007) observed that two isolates of *Salmonella* which did not phenotypically express resistance to aminoglycosides were harboring *aadA1* and *aadA2* genes, while Maynard et al. (2003) found that two *E. coli* isolates harboring the aph(3)-la gene, which confer resistance to Kanamycin and Neomycin, were susceptible to Kanamycin and Neomycin. Thus, our results and those of Ma et al. (2007) and Maynard et al. (2003) indicate that some antibiotic resistance genes are silent in bacteria *in vitro*; however, these silent genes can spread to other bacteria or turn on *in vivo*, especially under antibiotic pressure.

Furthermore, there were also discrepancies between the absence of the antibiotic gene test on the microarray and the phenotypic resistance (false negative). This was observed in isolates GO13-Goat (Am-C-Sxt-S-T-Amc); CA 9-Cattle (Am); and PL 18-Poultry *(*Am-C-Sxt-S). Resistance was phenotypically observed against the antibiotics written against each of the isolates but the genes were not detected by the microarray. This could be attributed to the non inclusion of the oligonucleotide probes encoding theses genes in the construction of the microarray or the genes encoding the resistance are novel. However, more research is needed in this area before conclusion can be established.

In conclusion, the microarray technique employed in this study proved to be an efficient method that allows for rapid detection and identification of resistance genes in *E. coli* isolates.

Table 4. The prevalence of chloramphenicol and qinolone resistance genes in *E. coli* isolates.

Gene	S O	U E S	O F	S A M	P L E
	Pig N=12	Goat N=10	Poultry N=8	Cattle N=10	Total N=40
cat4	00	01(10)	03(37.5)	01(10)	05(12.5)
catB2	00	00	00	00	00
catB3	00	00	00	00	00
catB8	00	00	02(25)	01(10)	03(7.5)
catI	00	01(10)	03(37.5)	01(10)	05(12.5)
catII	00	00	00	00	00
catP	03(25)	00	02	00	05(12.5)
cmlA	01(8.3)	010)	01(12.5)	02(20)	05(12.5)
cmlB	00	01(10)	02(25)	00	03(7.5)
floR	06(50)	05(50)	07(87.5)	04(40)	22(55.0)
intI1	04(33.3	03(30)	04(50)	04(40)	15(37.5)
intI2	01(8.3)	00	02(25)	01(10)	04(10)
qac delta E	07(58.3)	03(30)	06(75)	04(40)	20(50)
qnrA1	08(66.7)	05(50)	07(87.5)	04(40)	24(60)
qnrB	01(8.3)	00	00	00	01(2.5)
qnrS	00	00	00	01(10)	01(2.5)

N = Number of isolates hybridized.

Table 5. The prevalence of trimethoprim and sulfonamide resistance genes in *E. coli* isolates.

Gene	S O	U E S	O F	S A M	P L E
	Pig N=12	Goat N=10	Poultry N=8	Cattle N=10	Total N=40
dfrA1	00	01(10)	00	02(20)	03(7.5)
dfrA14	01(8.3)	03(30)	03(37.5)	00	07(17.5)
dfrA16	00	01(10)	01(12.5)	01(20)	03(7.5)
dfrA21	00	00	00	00	00
dhfrII	03(25)	00	01(12.5)	00	04(10)
dhfrV	03(25)	03(30)	03(37.5)	00	09(22.5)
dhfrVI	00	01(10)	00	00	01(2.5)
dhfrVII	00	00	02(25)	00	02(5)
dhfrXII	01(8.3)	00	01(12.5)	02(20)	04(10)
dhfrXIII	01(8.3)	00	01(12.5)	01(20)	03(7.5)
dhfrXV	00	00	00	00	00
Sul1	01(8.3)	01(10)	03(37.5)	02(20)	07(17.5)
Sul2	02(16.7)	04(40)	05(62.5)	03(30)	14(35)
sul3	01(8.3)	02(20)	00	02(20)	05(12.5)

N = Number of isolates hybridized.

Table 6. The prevalence of tetracycline resistance genes in *E. coli* isolates.

Gene	S O U E S O F S A M P L E				
	Pig N=12	Goat N=10	Poultry N=8	Cattle N=10	Total N=40
tet(C)	02(16.7)	01(10)	03(37.5)	02(20)	08(20)
tet(Y)	00	00	02(25)	00	02(5.0)
Tet30	00	00	01(12.5)	00	01(2.5)
TetA	05(41.7)	05(50)	04(50)	07(70)	21(52.5)
tetB	01(8.3)	05(50)	03(37.5)	00	09(22.5)
TetD	05(41.7)	02(20)	06(75)	02(20)	15(37.5)
TetE	04(33.3)	00	05(62.5)	02(20)	11(27.5)
tetG	01(8.3)	00	05(62.5)	00	06(15)
TetH	01(8.3)	00	01(12.5)	00	02(5.0)
TetJ	07(58.3)	04(40)	06(75)	02(20)	19(47.5)
TetK	01(8.3)	00	01(12.5)	00	02(5.0)
TetM	00	00	00	00	00
TetQ	00	00	00	00	00
TetS	00	00	00	00	00
TetT	00	00	02(25)	00	02(5.0)
TetV	00	00	02(25)	00	02(5.0)
TetW	00	00	00	00	00
TetX	00	00	02	00	02(5.0)

N = Number of isolates hybridized.

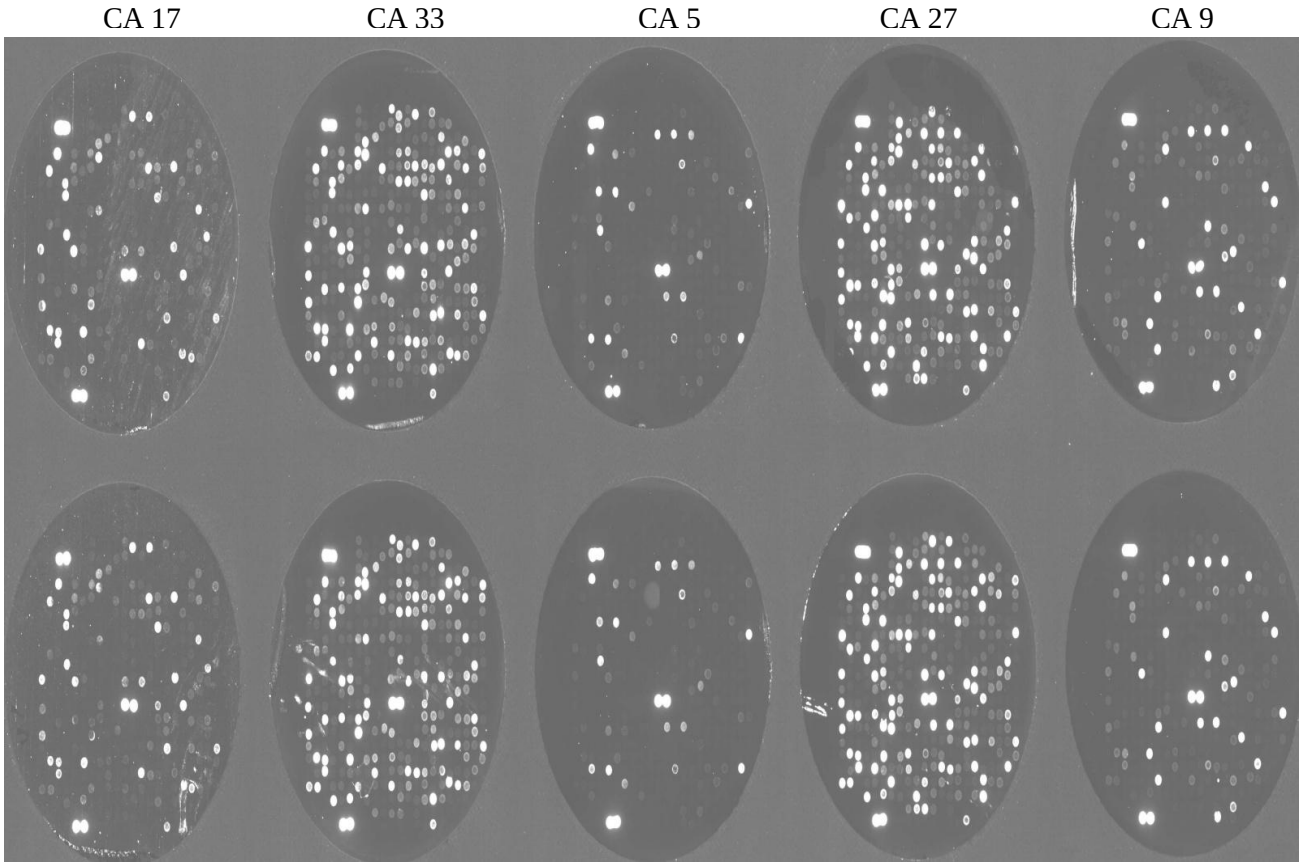

Figure 1. Microphotograph of microarrays hybridized with genomic DNAs of *E. coli* Isolates from cattle.

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