Journal of Advances in Microbiology

10(4): 1-8, 2018; Article no.JAMB.40666
ISSN: 2456-7116

Plasmid Profile and Multidrug Resistance in *Escherichia coli* Isolated From Swine in Aba Abia State Nigeria

Nwiyi, Paul Okechukwu[1*]

[1]Department of Veterinary Microbiology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMB/2018/40666
Editor(s):
(1) S. Pradeep, Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Kerala, India.
Reviewers:
(1) Vivek Kumar Singh, Nepal.
(2) O. Famurewa, Ekiti State University, Nigeria.
(3) Nazia Jamil, Punjab University, Pakistan.
(4) Tsaku Paul Alumbugu, Nasarawa State University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24716

Original Research Article

Received 2nd March 2018
Accepted 7th May 2018
Published 21st May 2018

ABSTRACT

Plasmid play a vital role in the emergence of multi-drug resistant bacteria. A total of 90 isolates of *Escherichia coli* was recovered from 100 swab samples (neonate, piglet and adult pigs). The isolates were identified based on morphological and biochemical classification. Twenty (20) of the representative isolates were confirmed using molecular method and then used for plasmid profile analysis. The isolates were tested for their antibiotic sensitivity and were found to be resistant to amoxicillin, oxacillin, erythromycine and streptomycin at 100%, 96%, 76% and 68% respectively. Multi-drug phenotypic characteristics were found in recovered bacteria. *Escherichia coli* (24.0%) exhibited resistant with the predominant resistance patterns of CIP-OXF-CET-CEZ-GEN-AUG-CXM. Plasmid cure was observed in some of the bacterial isolates which indicate that resistance was plasmid mediated while those that were not cured are chromosomally mediated. The study revealed that there were multidrug resistant strains of *E. coli* in Swine. Molecular detection of *E. coli* showed bands with amplicon size of 160bp. The plasmid showed poor visible band and the procedure may account for this. From the study, swine may serve as source of intermediate host for antimicrobial resistant.

**Corresponding author: E-mail: afodiokechukwu@yahoo.com;*

Keywords: Plasmid profile; molecular detection; multi-drug resistance; Escherichia coli, swine.

1. INTRODUCTION

Plasmids DNA are extra chromosomal element of limited size, usually stably inherited within a bacterial cell line and potentially capable of transfer between strains, species or genera [1]. They were first discovered in enteric bacteria, and from the late 1950s onwards were increasingly associated with antibiotic resistance. Its main function is to carry antibiotic resistance genes, which are responsible for increased pathogenicity of most bacteria [2,3]. The acquired resistance occurs both in endogenous flora of animals that were exposed to pathogenic and non - pathogenic bacteria [4,5,6]. These microorganisms may be shed in faeces causing contamination of soil, food and acquatic environments. Though administration of antimicrobial agents has been confirmed to be a successful way against bacterial contamination and infection, the widespread use has produced a reservoir of antimicrobial agents and MDR microorganisms. The occurrence and persistence of antimicrobial resistant bacteria in animals is accompanied by co-contamination of the environment leading to a great health challenge [7,8].

It has been observed that antibiotic susceptibility pattern of bacteria is not constant but dynamic and varies with time and environment [9]. According to Albinu et al. [10], *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim-sulfamethoxazole. The high incidence of drug resistant *E. coli* and other pathogens in our environment has made it needful for regular antibiotic susceptibility monitoring with the objective of making available alternative rational prescription and therapy [11]. The mechanisms of resistance involves polymorphisms in antimicrobial targets that reduce vulnerability, gene encoding, efflux system and proteins that fortify target sites or drug modifiers [12].

The property of multidrug resistance could be transferred via conjugation from resistant strains of *E. coli* to another by means of plasmid which occurs in cytoplasm of the donor bacterium and this multiplies independently of the chromosomal DNA [13,14]. Thus, a new bacterium with resistance factor emerges that is resistant to one or more antimicrobial agents [15]. Factors responsible for resistance can be transferred via mobile genetic elements, such as plasmids, transoposons or prophages thereby making it possible for horizontal transfer within and between bacteria species [16], especially in environments such as the gut microbiome [17]. *E. coli* is a member of the family enterobactericeae, which includes many genera, such as *Salmonella*, *Shigella* and *Yersinia*. Though *E. coli* is a normal flora of healthy animals, it has also been implicated as being responsible for antibiotic resistance genes [17]. Also, resistance transfer has been shown to occur between different species of farm animals and their environment [18].

The present study was conducted to isolate, detect and evaluate the antimicrobial resistance pattern of *E. coli* isolated from swine in Abia State and also to determine the drug of choice against *E. coil*.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Sterile swab sticks were used for collection of faecal swab. Samples were collected from different piggery farms located in Umungasi, Abayi, Osisioma, Ogbor hill, Umuojima and Ama-Ogbonna all within Aba North, Aba south Obi-ngwa, and Osissioma local government area of Abia State. A cross sectional survey study was employed. Random sampling method was adopted in choosing the swine from each pen. A total of 100 samples were used for the study. Samples collected were transported to the veterinary laboratory of Michael Okpara University of Agriculture for processing.

2.2 Identification of Isolates

The samples were cultured as described by Quinn et al. [19] on blood agar, MacConkey agar and Eosin Methylene Blue agar within 4 hrs of sample collection. The cultures were incubated at 37°C for 24 hrs. The colony obtained were sub-cultured onto Eosin Methylene Blue agar and incubated overnight to obtain a pure culture of *E. coli*. The colonies in the culture were counted in a colony counter. Pure growth of the isolate in a count of ≥120 colony forming units (CFU) of each faecal swab after incubation at 37°C for 24 hr was considered significant. A combination of colonial morphology, motility tests and Gram staining was conducted to determine the cultural characteristics of *E. coli*. The biochemical test conducted as described by

Cheesbrough (2004) was employed [20] and included; Catalase test, Oxidase test, oxidation-fermentation tests and hydrolysis of arginine.

2.3 Antiobiotic Susceptibilty Testing

The study made use of commercially available antibiotic discs (Oxoid, Basingstoke, United Kingdom) to evaluate the susceptibility pattern of the isolates. Twelve different antibiotics disc with various concentrations was used which includes augmentin (30 µg), oxfloxacin (5 µg), ceftriazone (30 ug), ciprofloxacin (5 µg), tetracycline (30 µg), streptomycin (10 µg), erythromycine (10 µg), gentamycin (30 µg), amoxicillin (25 µg), oxacillin (1 µg),ceftazidium (30 µg) and cefixime (5 µg). Disc diffusion method as described by Bauer et al. (1966) was employed [21]. After incubation, the zone of inhibition was measured while the Clinical Laboratory and Standards Institute [22] were used for the interpretation. *E. coli* ATCC25922 was used as control for comparing zone of inhibition.

2.4 Plasmid DNA Isolation and Profiling

Escherichia coli strains isolated were inoculated on Nutrient agar and incubated at 37°C for 24 hr. Alkaline lysis method (Zymogen UK) was employed for the plasmid DNA extraction. Three colonies of *E. coli* were picked and centrifuged and the supernatant was discarded. Three different buffers (B_1, B_2 and B_3) with buffer B_1 made up of 400mM Tris, 200mM Na EDTA, acetic acid with pH adjusted to 8.0; buffer B_2 made up of 3M Na, acetic acid with pH adjusted to pH 5.5 and buffer B_3 made of 10mM Tris, 2M Na EDTA, acetic acid with pH adjusted to 8.0, and lysing solution made of 4% SDS and 10mM Tris, were added to the pelleted cells at various times and thoroughly mixed by vortex before centrifugation at 16,000 xg for 2-3 minutes. The supernatant was loaded inside the zymo-spin column and centrifuged for 30 seconds and the flow through was discarded while W_1 buffer containing 200 µl was introduced into the column via a collection tube and centrifuged for 30 seconds. Thereafter, plasmid W_2 buffer 400 µl was added and centrifuged for 60 sec. The procedure as described by Ranjbar, [23] was employed. The spin column was introduced into a new micro centrifuge tube and 50 µl of DNA E_1 buffer was added and centrifuged for 30-40 seconds. The extracted DNA was electrophoresed on a 0.8% agarose gel stirred with ethidium bromide. The molecular study was conducted in Lahore Medical Research Centre and Diagnostic Laboratory, Benin.

2.5 Amplification of Target DNA

The target DNA was amplified by the polymerase chain reaction (PCR). The procedure employed by Promega cooperation, Madison USA was used in a volume of 25 µl containing genomic DNA from each *E. coli* isolate. The sequence of a pair of primer specific for the genus *Escherichia* (nuc-1 F-5-TCAGCAAATGCATCACAAACAG-3 and R-5-CGTAAATGCACTTGCTTCAGG-3), obtained from Inqaba Biotechnical Industries South Africa was used. Amplification was performed in Techne TC512 thermocycle with the cycling conditions, melting temperature and timing according to the manufacturers instructions.

2.6 Preparation of 0.8% Agarose Gel

The procedure as described by Bikandi et al. (2004) was employed [24]. A 0.8 g was dissolved in 100 ml Tris EDTA buffer to obtain 0.8% agarose gel was prepared. The mixture was heated for 3-5 min in a microwave to completely dissolve and allowed to cool to 56°C before addition of 8 µl of ethidium bromide. The agarose gel was poured into the electrophoresis chamber and allowed to solidify.

2.7 Plasmid DNA Detection

This was conducted by loading the first well with 10 µl of the molecular markers while the second well was loaded with 2 µl of loading dyes mixed with 8 µl of the plasmid DNA extract. Both were subjected to electrophoresis in a horizontal tank at 90v for 60 min. After electrophoresis, plasmid DNA bands were visualized by fluorescence ultraviolet light transilluminator and analysed using a photo documentation system. The molecular weights were estimated using molecular weight standard of the maker.

2.8 Plasmid DNA Curing

The plasmid cure was carried out according to the procedure described Akingbade [25], in which 9ml of freshly prepared nutrient broth was inoculated with 1 ml of pure *E. coli* culure that were grown in Luria-Bertanii broth containing antibiotics and incubated at 37°C for 24 hr. Then 1 ml of 10% sodium dodeocyl sulphate (SDS) curing agent was added and incubated at 37°C for 24 hr. The zone of inhibition was read and recorded and compared with the earlier antibiotic susceptibility test.

3. RESULTS

A total of 100 samples was collected and plated on Eosin methylene Blue agar. Colonies of bacteria that have greenish metallic sheen characteristics of *E. coli* were used to confirm the organisms. Table 1 shows the distribution patterns and isolation rate of *E.coli* from pigs of various age ranges. The highest isolation rate of *E. coli* was recorded in neonates with isolation rate of 93.3%, while adult pigs (above 12 mths) had the lowest isolation rate of 85.7%. The prevalence isolation rate was from 85.7% to 93.3% with an overall prevalence of 90.1%. This may suggest that the immunity of the neonates could be lower, while the adult pig is more resistance to *E, coli* organism. The antimicrobial susceptibility profiles of 50 *Escherichia coli* isolates obtained from swine is presented in Table 2. The spectrum of antimicrobial resistance in descending order for 12 antibiotics were amoxicillin, oxacillin, erythromycin, streptomycin, tetracycline, augmentin, cefixime, oxfloxacin, ceftriaxone, ceftazidime, ciprofloxacin and gentamycin. All the isolates were found resistant to amoxicillin while 96%, 76% and 68% were resistant to oxacillin, erythromycin and streptomycin, respectively. *Escherichia coli* isolate coded 21N-32N were positive for *E. coli* and this was visible in the bands at 160bp (Fig. 1).

Table 1. Isolation rate of *E.coli* from neonates, piglets and Adult-pigs in Abia State

Age range	Isolation rate (n)	% Isolation
Neonate (2-7 days)	28 (30)	93.3
Piglet (3-6 months)	32 (35)	91.4
Adult Above 12 months	30 (35)	85.7
Total	90 (100)	90.0

Table 2. Antibiotic susceptibility profile of *Escherichia coli* isolates obtained from swine

Antibiotic types	No and % resistance (n=50)
Ciprofloxacin (5 µg)	4 (8)
Oxfloxacin (55 µg)	6 (12)
Cefixime (5 µg)	12 (24)
Ceftriaxone (30 µg)	5 (10)
Ceftazidime (30 µg)	4 (8)
Amoxicillin (25 µg)	50 (100)
Augmentin (30 µg)	30 (60)
Oxacillin (1 µg)	48 (96)
Gentamycin (30 µg)	0 (0)
Erythromycin (10 µg)	38 (76)
Tetracycline (30 µg)	32 (64)
Streptomycin (10 µg)	34 (68)

No=number, %=percentage

Table 3. Antimicrobial resistant pattern of *Escherichia coli* isolated from swine

Isolates	Antibiotics resistance pattern	No of Patterns
N5	AUG-TET-CXM-CIP-OXF	5
N7	CET-CEZ-CXM-OXA-OXF	5
N16	CXM-CIP-OXF-TET-OXA	5
N18	CXM-CIP-OXF-STR-ERY-AUG	6
N19	GEN-OXF-TET-ERY-AMX	5
N20	CXM-TET-STR-CEZ-AMX	5
N21	OXA-GEN-CEZ-STR-ERY-OXF	6
N22	CET-AUG-ERY-AMX-OXA-OXF	6
N23	CXM-AUG-TET-STR—OXF-GEN	6
N24	AUG-CIP-TET-AMX-CXM-GEN	6
N25	CET-AUG-OXF-STR-AMX-TET-OXA	7
N26	CXM-CEZ-ERY-TET-AUG-STR-OXA	7
N27	CIP-TET-STR-AMX-GN-AUG-OXF	7
N28	CEZ-STR-CIP-OXF-OXA-CET-OXF	7
N29	CXM-ERY-AMX-TET-STR-OXF-CIP	7
N30	AMX-AUG-OXA-ERY-TET-STR-CIP	7
N32	CIP-OXF-CET-CEZ-CEZ-GEN-CXM-CIP	8
N33	AMX-TET-STR-OXF-OXA-CEZ-OXA-CXM	8
N36	AMX-TET-OXF-AUG-OXA-CEZ-GEN-CXM	8

AUG= Augmentin, TET = Tetracycline, CXM = Cefurixime, CIP = Ciprofloxacin, CET = Ceftriaxone, CEZ = Ceftazidim, OXF = Oxfloxacin, OXA = Oxacillin, Ery = STR Erythromycin, = Streptomycin, GEN = gentamycin, AMX = Amoxicillin

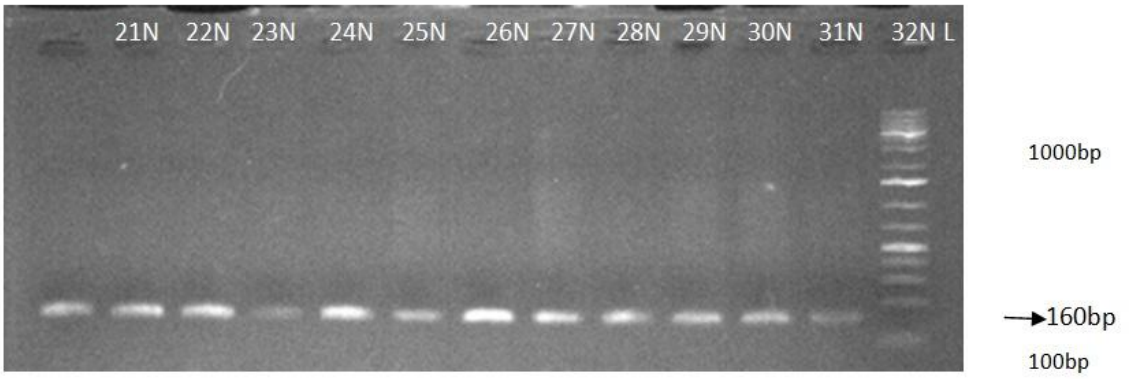

Fig. 1. L is 100-1000bp DNA ladder (molecular marker). Bacterial isolates of samples 21N-32N positive for *E.coli* with bands at 160bp using PCR

Table 4. Plasmid profile of multidrug resistant *Escherichia coli* isolates

Variables	No with plasmid (n=10)
Neonates (2-7 days)	10
Weaned (3-6 mths)	10
Adult (above 12 mth)	10

No=number, %=percentage N21, N22 up to N32 were coding systems used for identification of isolates present in the Fig. 1.

E. coli organism was resistant to several antibiotics which ranged from 60%-100%. The isolates from neonates were most sensitive to amoxicillin, oxacillin, ciprofloxacin, ceftazidine and erythromycin ($p<0.05$). Sensitivity to ceftazidime, ciprofloxacin and ceftriaxone was significantly high in isolates obtained from adult pigs above 12 months of age ($p<0.05$).

Table 3 shows antibiotic resistance pattern of *E. coli.* There were 19 resistance patterns in this study with 3 eight resistant patterns, 6 seven resistant patterns, 5 six resistant patterns and 5 five resistant patterns respectively. Table 4 shows Plasmid curing of multidrug resistant *E. coli* to detect whether the antibiotic resistance was as a result of plasmid or chromosomal effect. Fig. 1 shows the molecular confirmation of *E. coli*. Ten *E. coli* isolates were amplified and showed marked bands with amplicon size of 160bp while two isolates 24N and 32N were poorly amplified.

4. DISCUSSION

Escherichia coli is a common occurrence in the normal flora of most animal host, but could become pathogenic when the population present in the gastrointestinal tract is higher than necessary. The high isolation rate of *E. coli* in this study may be due to poor hygienic practices. Resistance commonly observed among the class of penicillin and macrolides and the sensitivity to the class of aminoglycoside, cephalosporins and quinolones as observed in this study is in agreement with Fontana et al. [26]. This may be most likely due to the presence of cephalosporinase and penicillinase enzymes which prevent the action of the Beta-lactam ring structure of the antibiotics and this is in agreement with Livermore and Iqbal et al. [27,28]. High resistance of *E. coli* to antibiotics like augmentine, amoxicillin and erythromycin is in disagreement with Farooqi et al. [29]. *E. coli* resistance to the Quinolones in this study was 10% and is in agreement with [27]. This may be due to *E. coli* organisms using different mechanisms to develop resistance such as ability to modify the antibiotic target sites, presence of inhibitory enzymes, possession of efflux pumps, and acquisition of resistant plasmids and mutation of the drug receptor site. The high antimicrobial resistance shown in this study may be due to factors such as inappropriate usage of antibiotics and this is in agreement with Eduardo et al. [30]. The amplified DNA with bands at 160bp confirms the isolates were *E. coli.* This could be due to the technique that was used, primer type and source.

The 19 resistant patterns observed in this study with the isolates displaying resistance to 3 or more classes of antibiotics is suggestive of multidrug resistance of *E. coli*. This observable difference in resistance patterns as shown in *E.*

coli from swine of different age range and locations may likely be due to other factors such as abuse of antibiotics. Gentamycin followed by ceftazidium were the drug of choice for the treatment of *E. coli* and this finding is consistent with Yah et al. [31]. *E. coli* resistance to ciprofloxacin and ofloxacine at 8% and 12% is in agreement with the report Oteo et al. [32] in Netherland and [33] in Germany.

The high resistance of *E. coli* to amoxicillin and tetracycline at (>64%) is closely related to that reported by Umolu et al. [34]. The post-curing susceptibility pattern of *E. coli* that are multi-drug resistant at pre-curing stage became susceptible. This suggests that plasmid-borne multidrug resistance gene had been denatured by the SDS used as the curing agent. Molecular confirmation evidence by amplification of bands with amplicon size of 160bp is closely related to the findings by Nathalie et al. [35], but at variance with Sadjie et al. [36], who confirmed amplicon size of 200bp. The differences in amplicon sizes may be due to different primer that was used. The isolates showed resistance to antibiotics but did not possess any plasmid, suggestive that chromosomal DNA may be responsible for carrying genes that confer resistance to antibiotics. Thus, treatment should be based on the result of laboratory.

5. CONCLUSION

Multidrug resistant *E. coli* was detected in swine. Most of the *Escherichia coli i*solates were sensitive to gentamycin, ciprofloxacin and ceftazidime whereas resistant to amoxicillin and oxacillin.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Alan V, Jesus M, Rober WJ, The role of plasmid in pathogenic bacteria: Mobile Arsenals. Journal of General Virology. 2001;147:4763-4780.
2. Datta N, Hughes VM. Plasmid of the Same Inc. Group in Enterobacteria before and after the medical use of antibiotics. Nature. 1983;306:613-616.
3. Piddock LJV. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic. Resistant bacteria that infect man and compromise antimicrobial chemotherapy? Journal Antimicrobial Chemotherapy. 1996;38:1-3.
4. Van den Boggaard AE. Antimicrobial resistance-relation to human and animal exposure to antibiotics. Journal Antimicrobial Chemotherapy. 1997;40:453-454.
5. Van der Bogaard AE, Stobberingh EE. Antibiotic usage in animal: Impact on bacterial resistance and public health. Drugs. 1990;58:589-607.
6. Grobbel M, Lubke-Becker A, Alesik E, Schwarz S, Wallmann J, Werckenthin C, Wieler LH. Antimicrobial susceptibility of *Escherichia coli* from swine, horses, dogs and cats as determined in the BFT GermVet monitoring program 2004-2016. Berl Munch TierarzH Wochensch. 2007;120:391-401.
7. Martinez JL. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proceedings Biological Science. 2009;276:2521-2530.
8. Hassan SH. Sensitivity of Salmonella and Shigella to antibiotics and chemo-therapeutic agents in Sudan. Journal Tropical Medicine and Hygiene. 1995;88:243-248.
9. Aibinu I, Aedripekun E, Odugbemi T. Emergence of quinolone resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State hospitals, in Nigeria. Nigeria Journal Health and Biomedical Science. 2004;3: 373-378.
10. Omiqie O, Enwani IB, Ohenhen RF, Umolu IP, Ben Edo-Osagie O. Bacteriological survey of wound infections in Benin-city, Nigeria. Nigeria Annual Natural Science. 2006;32:234-21.
11. Wright GD. The antibiotic resistance: The nexus of chemical and genetic diversity. Natural Review of Microbiology. 2007;5: 175-186.
12. Cole D, Drum DJ, Stalknecht DE, White DG, Lee MD, Ayers S, Sobsey M, Maurer JJ. Free-living Canada geese and antimicrobial resistance. Emergency Infections Disease. 2005;11:935-938.
13. Kozak GK, Boerlin P, Janecko N, Reid-Smith RJ, Jardine C. Antimicrobial resistance in *Esherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada. Applied

Environmental Microbiology. 2009;75:559-566.

14. Buxton A, Fraser G. Animal microbiology. Blackwell Scientific Publications, Oxford, London, Edinburg, Melbourne. 1977;1:85-86.
15. Amaechi N, Abbey ND, Achi OK. Plasmid profile and antimicrobial resistance ratings of *Escherichia coli* isolates from pigs and poultry in Abia State, Nigeria. International Journal of Current Microbiology and Applied Sciences. 2015;4:834-842.
16. Smillie CS, Smith MB, Friedman J, Cordero OX, David LA, Alam EJ. Ecology drives a global network of qene exchange connecting the human microbiome. Nature. 2011;480:241-244.
17. Marshall BM, Levy SD. Food animals and antimicrobials. Impacts on human health. Clinical Microbiology Review. 2011;24:718-733.
18. De Jong A, Thomas N, Simjee S, Godinho K, Sihryock TR. Pan-European monitoring of susceptility to human use antimicrobial agents in enteric bacteria Isolated from healthy food processing animals. Journal of Antimicrobial Chemotherapy. 2012;67: 638-651.
19. Quinn PJ, Carter ME, Markey BK, Cartel GR. Clinical veterinary microbiology. Mosby-year 1994. Europe Limited. Wolfe Publishing, London, England; 1994.
20. Cheesbrough M. District Laboratory Practice Manuel in Tropical Countries, Part 2 Cambridge University Press, New York. 2000;1:178-179.
21. Bauer AW, Kirby WMM, Sherris JC. Testing by a standard single disc method. American Journal of Clinical Pathology. 1966;36:493-496.
22. Clinical Laboratory and Standards Institute. Methods for disk diffusion: Approved Standard M2A8: Performance Standards for CLSI Antimicrobial Disc Susceptibility Tests; 2014.
23. Ranjbar R, Owlia H, Saderi H, Bameri Z, Izadi M, Jonaidi N, Morovati S. Isolation of clinical strains of bacteria harbouring different plasmids. Parkistan Journal of Biological Sciences. 2007;10:3020-3022.
24. Vivyan E, Hedges RW, Datta N. Two modes of curring transmissible bacterial plasmids. Journal General Microbiology. 1972;70:443-452.
25. Akingbade O, Balogun S, Ojo D, Akinduti P, Okerentugbe PO, Nwanze JC, Okonkwo IO. Resistant plasmid profile analysis of multidrug resistant *Esherichia coli* isolated from urinary tract infections in Abeokuta, Nigeria. African Health Science. 2014;14: 821-828.
26. Fontana R, Cornaglia G, Ligozzi M, Mazzriol A. The final goal: Penicillin-binding proteins and the target of cephalosporins. Clinical Microbiology and Infection. 2000;6:34-40.
27. Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clinical Microbiology, Reviews; 1995.
28. Iqbal M, Patel IK, Ain Q, Kiani Q, Rabbani KZ, Zaidi G, Mehdi B, Shah SH. Susceptibilty patterns of *Escherichia coli*: Prevalence of multi-drug resistant isolates and extended spectrum beta-lactamase phenotype. Journal Pakistan Medical Association. 2002;52:407.
29. Farooqi BJ, Shareeq F, Rizvi OK, Qureshi HS, Ashfaq MK. Changing pattern of antimicrobial susceptibility of organisms causing community acquired urinary tract infections. Journal Pakistan Medical Association. 2000;50:369-373.
30. Eduardo L, Airton DF, Jonas P, Pedro A. Prevalence of bacterial resistance in Surgical wound infections in peripheral arterial surgery. Journal Vascular Brasileiro. 2008;7(3).
    Available:http://idx.doi-org/10.1590/s 1677-54420-0800-300-009
31. Yah SC, Eghafona NO, Enebulele IO. Ampicillin usuage and Ampicillin resistant (AMPr) plasmids mediated *Escherichia coli* isolated from diarrheagenic patients attending some Teaching Hospital in Nigeria. Shiraz E-Medical Journal. 2006;7: 1-12.
32. Oteo J, Lazaro E, De-Abjo FJ, Baquero F, Campos J. Spanish members of EARSS antimicrobial-resistant invasive *Escherichia coli*, Spain. Emerging Infection Disease. 2005;4:546-553.
33. Alex B, Goesseri W, Schee CV, Margreet CV, Cornelissen J, Hubert E. Rapid emergence of oxfloxacin resistant *enterobacteriacae* containing multiple gentamycin resistant associated integron in a Dutch hospital. Emerging Infectious Disease. 2001;7:862-871.
34. Umolu P, Omigie O, Tatfeng Y, Omonogbe FI, Aisabokhale F, Ugbodagh OP. Antimicrobial susceptibity and plasmid

profiles of *Escherichia coli* isolates obtained from different human clinical specimens in Lagos, Nigeria. The Journal of American Science. 2006;2:70-75.

35. Nathalie YF, Ashok M, Wilfred C. Use of real-time polymerase chain reaction and molecular beacons for the detection of *Escherichia coli* 0157: H7. Analytical Biochemistry Journal. 2001;289:281-288.

36. Sadjia B, Roland B, Luke M, Gabrielle P, John F, Josee, H. Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. Journal of Clinical Microbiology. 2003;41: 2113-2125.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24716