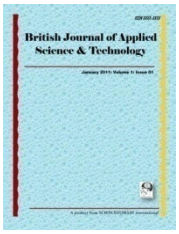

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Incidence of Multidrug Resistance R-plasmids among *Escherichia coli* Causing Urinary Tract Infections: A Case Study from Nigeria

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Case Study

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ABSTRACT

Aims: Study on the antimicrobial resistance pattern and plasmid screening of some clinical strains of *Escherichia coli* isolated from urine of some urinary tract infection out patients.
Study design: Cross-sectional study.
Place and Duration of Study: General hospital Lagos Island, Lagos, Nigeria, between March 2009 and August 2009.
Methodology: Fifty resistant strains of *E. coli* were isolated by standard procedures from 96 clinical specimens. Antimicrobial susceptibility testing and plasmid screening were done on the strains. Followed by plasmid isolation and gene transfer experiment. plasmid curing of the resistant plasmid was also carried out.
Results: Out of 96 samples screened only 50 (52.1%) yielded clinical isolates of *E.* coli. Among the various classes of antibiotic tested, high resistance was found with amoxycillin (100%), followed by tetracycline (98%), and augmentin (98%), while nitrofurantoin, ceftriaxone and ofloxacin being the most potent with (90%), (58%), (58%), sensitivity respectively. All the strains that were resistant to any antimicrobial agent were also resistant to amoxycillin. 34.4% of the isolates with multiple antimicrobial

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resistant haboured single plasmids ranging in sizes from 4.5kb to 4.8kb. Transformation experiment revealed that six of these resistant strains carried a common R-plasmid of size 4.8kb. Plasmid determined resistance to tetracycline was identified. **Conclusion:** This study has highlighted the emergence of multidrug resistance R-plasmids among *Escherichia coli* causing urinary tract infections in Nigeria. There is a high level of resistance to many antimicrobials that are frequently used in Nigeria. The uncontrolled use of antibiotics has contributed largely to this situation. Thus the government should make considerable effort to establish an antibiotic policy for the country.

Keywords: Antimicrobial susceptibility; R-plasmid; Escherichia coli; urinary tract infection;

1. INTRODUCTION

Community-acquired urinary tract infection (UTI) is one of the most common infectious diseases and a frequent cause of presentation of outpatient treatment. While mortality rates are not usually high the cost to the global economy is substantial (Bontein et al., 1990; Marquez et al., 2008; Vorland et al., 1985). Generally UTIs are mediated by gram –negative bacteria with the most common of these being *Escherichia coli* and *Klebsiella pneumonia*, but can also include *Acinetobacter* and *Enterobacter spp*. (Aibinu et al., 2004; Daini et al., 1995; Lipsky, 1989; Marquez et al., 2008; Panse and Wadhwa, 1973; Stelling et al., 2005). Management of the infection is becoming progressively complicated by the ongoing increase in resistance to antibiotics manifested by UTI causing organisms. Moreover multiple antibiotic resistance in bacteria has been ascribed to the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype (Blango and Mulvey, 2010; Daini et al., 1995; Diani et al., 2006; Hughes et al., 1981; Kruse and Sorum, 1994; Shirley et al., 2004). It has been shown that R-plasmids mediated antibiotic resistance can spread in a population subjected to heavy antibiotic therapy (Barker, 1999; Bonten et al., 1990; Kruse and Sorum, 1994; Ogbolu et al., 2011; WHO., 1983).

Furthermore the use of antibiotics perpetuated antibiotic resistant plasmids in communities like Lagos, Nigeria, where there is an unrestricted use of antimicrobial agents. Thus, this present work describes a study on the antimicrobial resistance patterns and plasmid screening of some *Escherichia coli* strains isolated from the urine of UTI outpatients, attending General Hospital Lagos Island, Lagos, Nigeria.

2. MATERIALS AND METHODS

2.1 Bacteriology

50 resistant strains of *Escherichia coli* isolated by standard procedures, (Barrow and Feltharni, 1993), from 96 clinical specimen sent to the Diagnostic Laboratory of Medical Microbiology and Parasitology of the General Hospital Lagos Island, Lagos, Nigeria, from March to August, 2009, were studied.

2.2 Antimicrobial Susceptibility Testing

Antimicrobial Disc Diffusion Tests were carried out as previously described by Daini and Adesemowo (2008), and standardized by the method of National Committee for Clinical Laboratory Standards, NCCLS, (2000). *Escherichia coli* ATCC25992,was used as control, while the following antibiotic discs were used: Amoxycillin 25µg,Nalidixic acid 30µg, Cotrimoxazole25µg,Nitrofurantoin 200µg,Augmentin 30µg,Tetracycline 25µg, Gentamicin 10µg, Ofloxacin 5µg, Cefotaxime 30µg,Ciprofloxacine 5µg, Ceftriaxone 30µg,Cefuroxime 30µg, Imipenem 5µg and Pefloxacin 5µg.

2.3 Isolation and Separation of Plasmid DNA

Plasmid DNA was isolated, separated and stained as previously described Olukoya et al., (1988).

2.4 Genetic Transfer

Transformation was done as described by Hanahan D. (1983), using *Escherichia coli* K-12,HB101 (ara-14, galk2, hsd520, lacyl, leu, mt 1o1,proA2, recA13,rspsl 20, supE44, thixyl-5) as recipient and plasmid pBR 322 as the positive control. Co-transformation resistant character was determined by testing all transformants against all antibiotics to which the donor strain was resistant, extracts from transformants were obtained as described above and subjected to Agarose gel electrophoresis. Transformation was confirmed as positive only when resistant transformants were shown to contain a plasmid of a size similar to that found in the original isolate.

2.5 Plasmid Curing

The curing of the resistant plasmids of the clinical *Escherichia coli* isolates was done as described by Vivyan et al. (1972).

3. RESULTS

All the 50 clinical *E. coli* strains isolated were resistant to most of the antimicrobial agents tested. 64% of these isolates showed multiple resistances to eight or more of the antimicrobial agents used. the frequency of susceptibility to nitrofurantoin was the highest (90%), while sensitivity to amoxycillin (0%) was the lowest (Table 1). All the resistant strains were resistant to amoxycillin and tetracycline respectively. Table 2, shows antimicrobial resistant patterns in relation to plasmid contents. Eleven distinct antimicrobial resistant patterns were identified among the resistant strains.

Of the 50 clinical resistant isolates screened, 34.4% harbored plasmids ranging in molecular sizes from 4.5kb to 4.8kb.Plasmids were not detected in 39 of the resistant strains indicating that their resistance was probably chromosomally borne. It is noteworthy that all the strains harboring plasmids were resistant to eight or more of the antimicrobial agents i.e. occurrence of multiple antimicrobial resistance. Amoxycillin and Tetracycline were common to almost all the antimicrobial susceptibility patterns (Table 2).

Also all the multiple antimicrobial resistant strains possessed only one plasmid each.

Transformation experiment showed that 54.5% of the resistant strains that harbored plasmids were able to transfer their resistance plasmids to *E. coli* K.-12 HB101.The R-plasmids isolated in this study have a common size of 4.8kb.

Plasmid-determined resistance to tetracycline was found (Table 3). All the strains harboring R-Plasmids were cured their plasmids upon treatment with sodium dodecyl sulphate with resultant loss of their plasmid –associated properties. This indicates that the antibiotic resistant genes of the bacteria strains used in this study were plasmid borne.

Table 1: Antibiotic sensitivity pattern of clinical isolates of *E. coli*

Antibiotics	Number sensitive	% Sensitive	% Resistant
Ceftriaxone	29	58	42
Imipenem	13	26	74
Cefotaxime	22	44	56
Cefuroxime	4	8	92
Nalidixic	20	40	60
Pefloxacin	27	54	46
Ciprofloxacin	22	44	56
Ofloxacin	29	58	42
Augmentin	1	2	98
Cotrimoxazole	8	16	84
Nitrofurantoin	45	90	10
Amoxycillin	0	0	100
Tetracycline	1	2	98
Gentamicin	28	56	44

Table 2: Antimicrobial resistance patterns of 50 clinical *E. coli* isolates in relation to plasmid contents

Antimicrobial Resistant Pattern	No showing pattern	% showing pattern	No with plasmids
Ipm Cfx Gen Nal Aug Tet Amx Cot	3	6	1
Ipm Ctf Cfx Aug Tet Amx Cot Pef	2	4	1
Ctx Ofx Cip Cfx Gen Nal Aug Tet Amx Cot Pef	2	4	1
Ipm Cip Cfx Gen Nal Aug Tet Amx	4	8	1
Ipm Ctx Ofx Cip Cfx Nal Aug Tet Amx Cot Pef	6	12	1
Ipm Ctx Ofx Cfx Cip Gen Nal Aug Tet Amx Cot Pef	8	16	1
Ipm Ctx Ofx Cip Cfx Nal Aug Tet Amx Cot Pef	2	4	1
Cro Ipm Ctx Ofx Cip Cfx Gen Nal Aug Tet Amx Cot Pef	5	10	1
Ofx Cip Cfx Gen Nal Aug Tet Amx Cot Pef	3	6	1
Cro Ipm Cip Cfx Gen Nal Aug Tet Amx Cot Nit	6	12	1
Cro Ipm Ctx Ofx Cip Cfx Gen Nal Aug Tet Amx Cot Nit Pef	9	18	1

Amx = Amoxycillin; Nal = Nalidixic; Cfx = Cefuroxime; Tet =Tetracycline; Cip = Ciprofloxacin; Ctx = Cefotaxine; Pef = Pefloxacin; Gen= Gentamicin; Cro = Ceftriaxone; Ipm = Imipenem; Ofx = Ofloxacin; Aug = Augumentin; Cot = Cotrimoxazole; Nit = Nitrofluratoin;

Table 3: Characteristics of some of the clinical bacterial R-plasmids

Bacterial strains	Plasmid Molecular Size (kb)	Antibiotic Gene Transferred to *E. coli* (HB101)	Transformant R-plasmid Size (kb)
Eco 14	4.8	Tet	4.8
Eco 18	4.8	Tet	4.8
Eco 44	4.8	Tet	4.8
Eco 27	4.8	Tet	4.8
Eco 38	4.8	Tet	4.8
Eco 3	4.8	Tet	4.8

4. DISCUSSION

All the *E. coli* strains reported here were isolated from patients with urinary tract infections, and 64% of these clinical isolates showed multiple resistance to eight or more of the antimicrobial agents tested. This result is similar to that obtained by Daini and Adesemowo (2008); Hughes et al. (1981) and Marquez et al., (2008). This study has revealed a very high level of resistance of the clinical strains of *E. coli* to Amoxycillin, Tetracycline and Augumentin respectively. This was in agreement with the findings of Aibinu et al., (2004); Daini and Adesemowo (2008); Ogbolu et al., (2011), Stelling et al., (2005). This may be due to the indiscriminate widespread use of these antibiotics in Nigeria. The high sensitivity of the clinical strains of *E. coli* to Nitrofuratoin, Ceftriaxone, Ofloxacin is similar to that reported by Blango and Mulvey (2010), Bonten et al. (1990); Daini and Adesemowo (2008); Diani et al., (2006). Resistance to high level of antibiotics has been ascribed in most instances to the presence of plasmids (Barker, 1999; Diani et al., 2006; Sherley et al., 2004). The most common plasmids encountered were 4.8kb in size. This is in agreement with the findings of Daini et al. (1995), Olukoya et al. (1988) and Sherley et al. (2004). 54.5% of the drug resistant strains carried R-plasmids. Plasmid determined resistance to tetracycline was found.

The emergence of R-plasmids in this study could be ascribed to the indiscriminate and widespread use caused by the over- the- counter availability of antibiotics as well as the higher exposure of people to enteric flora in places with poor sanitation (Aibinu et al., 2004; Marquez et al., 2008; Ogbolu et al., 2011; Olukoya et al., 1988). The use of transformation enabled us to detect non-transmissible plasmids. In view of the occurrence of multiple antibiotic resistant strains coupled with the re-emergence of the tetracycline resistant plasmids; considerable effort must be made to establish an antibiotic policy for the Hospital and the country.

5. CONCLUSION

This study has highlighted the emergence of multidrug resistance R-plasmids among *Escherichia coli* causing urinary tract infections in Nigeria. There is a high level of resistance to many antimicrobials that are frequently used in Nigeria. The uncontrolled use of antibiotics has contributed largely to this situation. Thus the government should make considerable effort to establish an antibiotic policy for the country.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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