Australian Journal of Basic and Applied Sciences, 2(3): 397-400, 2008
ISSN 1991-8178

Antimicrobial Susceptibility Patterns and R-Plasmids of Clinical Strains of Escherichia Coli

[1]O.A. Daini and [2]A. Adesemowo

[1]Department of Biochemistry, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Remo Campus, P.M.B. 2005, Ikenne, Ogun State, Nigeria.
[2]Dept. Of Medical Microbiology and Parasitology, O.A.C.H.S., Olabisi Onabanjo University, Remo Campus, P.M.B. 2005, Ikenne, Ogun State, Nigeria.

Abstract: A total of sixty-one resistant stains of *Escherichia coli* from 105 clinical specimens obtained from different body sites at the University College Hospital, Ibadan were subjected to antimicrobial susceptibility testing and plasmid profiling. Among the various classes of antibiotic tested, high resistance was found with Amoxicillin (90.16%), followed by tetracycline (88.52%) and co-trimoxazole (78.69%) while Nitrofurantoin and Ofloxacin being the most potent with (90.16%) and (54.10%) sensitivity. All the strains that were resistant to any antimicrobial agents were also resistant to amoxicillin. Plasmids ranging in molecular sizes from 0.12kb to 23.1kb were extracted from 40 (65.57%) of these isolates and grouped into seven plasmid profiles.Transformation experiment revealed that 40% of the resistant strains carried a common R – plasmid of size 23.1kb. Plasmid determined resistance to Amoxicillin was identified.

Key words: Antimicrobial susceptibility, plasmid profile, *Escherichia coli*

INTRODUCTION

Escherichia coli have been associated with a number of disease syndromes. Among these are often severe and sometimes fatal infections such as pclonephritis, septicemia, meningitis, endocarditis, urinary tract infection, epidemic diarrhoea of adults and children (Fine gold S.M. and Martins W.J. (Eds) 1982; Daini O.A., *et al.,* 2005).

Multiple antibiotic resistance in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype (Daini O.A., *et al.,* 1995; Olukoya D.K.,*et al.,* 1988). It has been shown that antibiotics therapy can select for antibiotic resistant strains in the faecal flora (Datta N., *et al.,* 1971) and R – plasmid mediated antibiotic resistance can spread in a population subjected to heavy antibiotic therapy (Daini O.A., *et al.,* 1995; Olukoya D.K., *et al.,* 1988). Furthermore the use of antibiotics perpetuated antibiotic resistant plasmids in communities like Nigeria where there is an unrestricted use of antimicrobial agents. Thus this paper describes the antimicrobial susceptibility patterns and plasmid screening of some resistant strains of *Escherichia coli* commonly isolated from clinical specimens in University College Hospital, Ibadan, Nigeria.

MATERIALS AND METHODS

Bacteriology:

Sixty-one resistant strains of *Escherichia coli* isolated by standard procedures (Barrow G.I., and Feltharni R.K.A. 1993) from 105 Clinical Specimens sent to the Diagnostic Laboratory of Medical Microbiology and Parasitology Laboratory of University College Hospital, Ibadan from March to August 2006 were studied.

Antimicrobial Susceptibility Testing:

Antimicrobial disc susceptibility tests were carried out on the isolates using stokes disc diffusion technique (Ewing, 1986, Stokes F.J. and Regway G.L. 1987) on freshly prepared mueller – Hinton agar (Oxoid, England) and standardized by the method of National Committee for Clinical Laboratory Standard (II) using the following antibiotic discs; Amoxicillin 25µg, tetracycline 30µg, cotrimoxazole 30µg, Ofloxacin 30µg, nitrofurantoin 300µg, nalidixic 30µg, gentamicin 10µg, Augmentin 30µg.

Corresponding Author: O.A. Daini, Olabisi Onabanjo University, Remo Campus, P.M.B. 2005, Ikenne, Ogun State, Nigeria.

Escherichia coli NCTC10418 was used as control. Plates with antibiotic disc were incubated for 24 hours at 37°C and sensitivity pattern was compared with that of the control organism.

Isolation and Separation of Plasmid DNA:

Plasmid DNA was isolated, separated and stained as previously descended by Takahashi and Nagano (Takahashi S. and Nagano Y. 1984). Plasmid profile groups were constructed by grouping strains possessing the same profile (containing the same number and molecular mass) or part of a profile constituting a core profile. Bacterial strains that carried the plasmid were regarded as constituting one plasmid group.

Genetic Transfer:

Transformation was done as described by Hanahan (1983) using *Escherichia coli* K – 12, HB 101 (ara – 14, galk2, hsd 520, lacyl, leu mt101, proA2, recA13, rsps120, supE44, thii xy1 – 5) as recipient and plasmid pBR322 as the positive control. Co – transformation resistant character was determined by testing all transformants against all antibiotics to which the donor strains 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 (s) of a size similar to that found in the original isolate.

Plasmid Curing:

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

RESULTS AND DISCUSSION

Results:

Of the 105 Clinical *E.coli* strains isolated, 61 were resistant to most of the antimicrobial agents tested. The frequency of susceptibility to Nitrofurantion was the highest (90.16%) while sensitivity to Amoxicillin (9.84%) was the lowest (Table 1). All the resistant strains were resistant to amoxicillin. Of 61 clinical resistant isolates screened, 40 (65.57%) harboured plasmids ranging in molecular weight from 0.12kp to 23.1kbp. Plasmids were not detected in 21 of the resistant strains indicating that their resistance was probably chromosomally borne. Eight different plasmid profile groups for the antibiotic – resistant strains could be defined. The number of strain per plasmid profile group vary from 1 – 9 (Table 2).

Table 1: Antibiotic Sensitivity pattern of Clinical Isolates of Escherichia coli

Antibiotics	Number Sensitive	% Sensitive	% Resistant
Nitrofurantoin	55	90.16	9.84
Ofloxacin	33	54.10	45.90
Gentamicin	28	45.90	54.10
Nalidixic	26	42.62	57.38
Augmentin	14	22.95	77.05
Cotrimoxazole	13	21.31	78.69
Tetracycline	7	11.48	88.52
Amoxicillin	6	9.84	90.16

Table 2: Plasmid profile groups of antibiotic resistant clinical strains

Plasmid Profile	No of Strains	Molecular mass (kb) of plasmids
0	21	No Plasmids
1	9	23.1
2	8	23.1, 0.82, 0.56
3	7	23.1, 0.82, 0.56, 0.12
4	7	23.1, 0.12
5	6	23.1, 0.56, 0.12
6	2	23.1, 0.56
7	1	23.1, 0.82, 0.12

The most common antimicrobial resistance pattern was OflGenNal AugCotTetAmx. This was followed in decreasing order of occurrence by the R – types resistance patterns: AugCotTetAmx, AugTetAmx, GenAugCotTetAmx, GenNalAugCotTetAmx, TetAmx, GenNalAugCotAmx, CotTetAmx, NalAugAmx, NitGenNalCotTetAmx, OflGenNalCotTetAmx, OflNalAugCotTetAmx, AugCotTet, OflNalCotTet and NitOflGenNalAugCotAmx. (Table 3). Strains showing the resistance pattern. OflGenNalAugCotTetAmx harboured the highest number of plasmids while the lowest number was found in the resistance patterns TetAmx, NalAugAmx, AugCotTet, CotTetAmx, OflNalCotTet, NitOflGenNalAugCotAmx respectively.

Table 3: Antimicrobial Resistance Patterns of 61 Clinical isolates of E. coli in relation to plasmid contents.

Antimicrobial Resistance Patterns	No showing Pattern	%	No with Plasmids
TetAmx	4	6.56	1
NalAugAmx	3	4.92	1
AugCotTel	2	3.28	1
AugTetAmx	6	9.84	3
CotTetAmx	4	6.56	1
OflNalCotTet	1	1.64	1
AugCotTetAmx	7	11.48	3
GenAugCotTetAmx	5	8.20	3
OflNalAugCotAmx	2	3.28	2
OflNalCotTetAmx	2	3.28	2
GenNalAugCotAmx	4	6.56	2
NitGenNalCotTetAmx	2	3.56	2
OflGenNalCotTetAmx	2	3.56	2
OflNalAugCotTetAmx	2	3.56	2
GenNalAugCotTetAmx	5	8.20	4
NitOflGenNalAugCotAmx	1	1.64	1
OflGenNalAugCotTetAmx	9	14.76	9

KEY: Amx = Amoxicillin, Tet = Tetracycline, Cot = Cotrimoxazole, Aug = Augumentin, Nal = Nalidixic, Gen = Gentamicin, Ofl = Ofloxacin, Nit = Nitrofurantion

Table 4: Charcteristic of some of the clinical bacterial R - Plasmids

Bacterial Strain	Plasmids Mol. Size (kb)	Antibiotic gene transferred to E.coli	Transformant R-plasmid size (kb)
Eco 2	23.1, 0.82, 0.56	Amx	23.1
Eco 3	23.1, 0.82, 0.56, 0.12	Amx	23.1
Eco 5	23.1, 0.56, 0.12	Amx	23.1
Eco 16	23.1, 0.82, 0.56	Amx	23.1
Eco 17	23.1, 0.82, 0.12	Amx	23.1
Eco 21	23.1, 0.56	Amx	23.1
Eco 24	23.1, 0.82, 0.56, 0.12	Amx	23.1
Eco 26	23.1, 0.82, 0.56	Amx	23.1
Eco 28	23.1, 0.82, 0.56, 0.12	Amx	23.1
Eco 32	23.1, 0.82, 0.56	Amx	23.1
Eco 35	23.1, 0.82, 0.56, 0.12	Amx	23.1
Eco 37	23.1, 0.82, 0.56	Amx	23.1
Eco 38	23.1, 0.82, 0.56, 0.12	Amx	23.1
Eco 41	23.1, 0.82, 0.66	Amx	23.1
Eco 46	23.1, 0.56, 0.12	Amx	23.1
Eco 47	23.1, 0.82, 0.56, 0.12	Amx	23.1

Transformation experiment showed that 40% of the resistant strains that harboured plasmids were able to transfer their resistance plasmids to *E.coli* K – 12 HB101. Plasmid – determined resistance to amoxicillin was found. It is noteworthy that all the R – plasmids isolated in this study have a common molecular size of 23.1kb (Table 4).

All the strains harbouring R – Plasmids were cured of their plasmids upon treatment with sodium dodecyl sulphate (SDS) with resultant loss of their plasmid – associated properties. This indicates that the antibiotic resistant genes of the bacterial strains used in this study were plasmid borne.

Discussion:

Antimicrobial resistance patterns revealed a total of seventeen patterns (Table 3). All the resistant strains were resistant to Amoxicillin.

This study has revealed that resistance of clinical strains of *E.coli* to amoxicillin and tetracycline is on the increase in accordance with the work done by Daini *et al* (1995), Hsueh *et al* (2002). This may be due to the indiscriminate widespread use of these antibiotics in the country. It is also noteworthy that these patterns depict the occurrence of multiresistant strains. This is similar to that obtained by Olukoya *et al* (1988,1993).

Resistance to high level of antibiotics has been ascribed in most instances to the presence of plasmids (Daini O.A., *et al.,* 1995; Foster T.J., 1983). The most common plasmids encountered were 23.1kb in size. This is in agreement with the findings of Olukoya *et al* (1993), Daini *et al* (1998). 40% of the drug resistant strains carried R – plasmids. Plasmid determined resistance to amoxicillin was found. A different plasmid profile could be seen for each of the 16 R – plasmids and plasmids of the same molecular weight could be found in different strains. Thus the plasmid profile of these strains was diverse in nature. Plasmid profiling analysis distinguished more strains than the antimicrobial susceptibility patterns in agreement with the findings of Daini *et al* (1995), Levy *et al* (1985).

The transformation experiment enabled us to detect non – self transmissible plasmids while curing of the resistant strains of the R – plasmids with SDS showed that their antimicrobial resistant genes were plasmid borne (Daini O.A., *et al.,* 1995; Vivyan E., *et al.,* 1972).

The emergence of R – plasmids particularly against drug used for first – line therapy could be due to lapses from institutional monitoring policies and antibiotic prescribing policy as many physicians in Nigeria usually prescribe without recourse to antibiotic sensitivity patterns (Montefiore D., *et al.,* 1989; Ogunsola F.T., *et al.*, 1997).

Our study has highlighted diverse plasmid profiles and widespread antimicrobial resistance patterns among the clinical isolates and *Escherichia coli* from Nigeria and we hope that this information from this locality would be a useful baseline for further epidemiological studies.

AKNOWLEDGEMENT

The authors are grateful to the staff of the diagnostic laboratory of Medical Microbiology and Parasitology Laboratory of University College Hospital, Ibadan for their assistance in the collection of samples.

REFERENCES

Fine gold, S.M. and W.J. Martins (Eds), 1982. Genus Escherichia in Diagnostic Microbiology Mosby Publishing Company London 6th Edition pp: 222-224.

Daini, O.A., D.K. Olukoya and A.A. Ogunjimi, 1995. Genetic Analysis of Tetracycline – Resistant Plasmids in Euteropathogenic E. Coli isolated from Patients in Nigeria J. Diarrhoeal Dis Res., 13: 39-43.

Daini, O.A., A. Ogunledun, K.T. Lawal, F.K. Moibi, A. Odunowo and O. Ogunwobi, 1998. Plasmid – borne *strep to mycin* Resistance of *Escherichia coli* in Sagamu, Nigeria. Afr. J. Med. Pharm. Sci., 1: 18-23.

Daini, O.A., O.D. Ogbolu and A. Ogunledun, 2005. Quinolones Resistance and R – plasmids of some Gram negative enteric Bacilli. Afr. J. Clin and Exp. Micro., 6: 14-19.

Levy, S.B., R.W. Hegdes, F. Sullian, A.A. Madaros and H. Sosrosephiro, 1985. Multiple Antibiotic resistance plasmids in enterobacteriaceae isolated from diarrhoea specimens of hospitalized children in Indonesia. Antimicrob. Chemother, 16: 7-16.

Olukoya, D.K., O. Daini, S.A. Alabi, O.A. Coker, T.O. Odugbemi and E.O. Akinrimisi, 1988. Antimicrobial resistance patterns and plasmids of enteropathogenic *Escherichia coli* isolated in Nigeria. Eur. J. Epidermiol, 4: 304-309.

Datta, N., M.C. Faiers, D.S. Reevers, W. Brumfitt, F Orskow and I. Orskov, 1971. R-factors in *Escherichia coli* in faeces after oral chemotherapy in general practice. Lancet, ii: 312-315.

Barrow, G.I. and R.K.A. Feltharni, 1993. Characters of Gram – negative bacteria. In: Cowan and Steel Manual for Identification of Medical Bacteria. 3rd Edition Cambridge University Press, pp: 94-149.

Ewing, W.H., 1986. Edwards and Ewings Identification of Enterobacteriaceae 4th Edition Elsevier Science Publishing, New York.

Stokes, F.J. and G.L. Regway, 1987. Clinical Microbiology 6th Edition, Edwards Arnold, London.

National Committee for Clinical Laboratory Standards (NCCLS), 2000. Performance standard for antimicrobial susceptibility testing, 10th Information Suppliment approved standard, M100 – S10, Wayne P.A.

Takahashi, S. and Y. Nagano, 1984. Rapid procedure for isolation of plasmid DNA and application to epidemiological analysis. J. Clin, Microbiol, 20: 608-613.

Hanahan, D., 1983. Studies in Transformation of *Escherichia coli* with plasmids. J. Mol, Biol., 166: 557-580

Vivyan, E., R.W. Hedges and N. Datta, 1972. Two modes of curing transmissible bacterial plasmids. J. Gen. Microbiol, 70: 443-452.

Hsueh, P.R., C.Y. Liu and K.T. Luh, 2002. Current Status of Antimicrobial Resistance in Taiwan. Emerging Infect. Dis., 8: 132-135.

Olukoya, D.K., O.A. Daini and M. Niemogha, 1993. Preliminary epidemiological studies in Tetracycline resistance plasmids isolated from entric bacteria in Nigeria. Trop. Georg. Med., 45: 117-120.

Foster, T.J., 1983. Plasmid – determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbial Rev., 47: 361-409.

Montefiore, D., O. Rotimi, F.A.B. Adeyemi – Duro, 1989. The problem of bacterial resistance to antibiotics among strains isolated from hospital patients in Lagos and Ibadan, Nigeria. J. Antimicrob. Chemother, 23: 641-651.

Ogunsola, F.T., C.N. Kesah and T. Odugbemi, 1997. Antimicrobial resistance in Nigeria, an overview Nig. Qt J. Hosp. Med., 7: 57-61.