Ampicillin Usage and Ampicillin Resistant (Ampr) Plasmids Mediated Escherichia Coli Isolated from Diarrheagenic Patients Attending Some Teaching Hospital in Nigeria.

Yah SC*, Eghafona NO**, Enabulele IO**, and Aluyi HSA**.

*Department of Medical Microbiology and Parasitology, Madonna University, Okija, Nigeria,** Department of Microbiology, University of Benin, Benin City, Nigeria

Correspondence: Yah. Clarence, Dept of Medical Microbiology & Parasitology, Madonna University, Okija, Anambra, State, Nigeria, Email: yahclar@yahoo.com, Phone:+234(806)341-8265.

Abstract:

From 2001 to 2004, we carry on a survey on the utilization of ampicillin among some diarrheagenic patients attending some Teaching Hospitals in Nigeria. From the result 723(58.2%) volunteers responded to the questionnaires, where only 83(11.4%) consult a doctor for prescription, 397(54.9%) from drug retailers while 142(20.1%) self prescribe and 56(7.7%) prescription was base on the advice of friends and relatives. The results also indicate that 229 (31.7%) take full regimen if prescribed by a doctor and 494(68.3%) stop the consumption of the drug when the signs and symptoms subside. Of the 249 diarrheagenic clinical isolates of E coli strains obtained from 2001 to 2004, 78 (31.3%) were obtained from UBTH, 41(16.5%) from ABUTH, 38(15.3%) from NAUTH and 92(36.9%) from UCH with a mean average of 63(25.3%). Among the antibiotics tested, ampicillin was the least susceptible antibiotics while the quinolones were the most susceptible. Most of the E coli strains screened had one or more resistant R plasmids. 52% of the E coli harbour ampicillin resistant plasmids of different molecular weights that ranged from ≤0.451kbp to ≥1.254kbp. Mostly Chromosomal DNA mediated most of the E coli isolates resistant to tetracycline, erythromycin, and ciprofloxacin. None of the Teaching Hospital had a surveillance method for evaluating the emergence of resistance pathogens.

1

Introduction:

Escherichia coli is an important opportunistic pathogen that has shown an increasing antimicrobial resistant to most antibiotics (Winokur et al., 2001; Miranda et al., 2004) isolated from humans and animals (Poppe et al., 2005; Sayah et al., 2005). It is a gram negative bacillus that lives in the intestinal tract of virtually everyone and usually become opportunistic pathogens when it gain access to certain parts of the body such as urinary tract, bloodstream and wounds, where it spread causing various infections (Prescott et al., 2003). Intestinal strains of E coli are primary cause of urinary tract infections, septicemia, diarrhea, neonatal meningitis and nosocomial infections. Individuals who are debilitated or have other predisposing factors are at much higher risk for infection than healthy person (Lisa and Rodgers, 1999). E coli have been reported to be the leading cause of diarrhea diseases in addition to pathogens such as Salmonella, Shigella, Yersinia, Vibrio, Campylobacter species Entamoeba histolytica, and Giardia lamblia in developing countries (Prescott et al., 2003). E coli strains causing diarrhea world wide, mostly in infants include; entero-pathogenic E. coli (EPEC), entero-toxigenic E coli (ETEC), entero-hemorrhagic E coli (EHEC), entero-invasive E coli (EIEC), entero-aggregasive E coli (EAEC) and entero adherent E coli (EAEC) or diffusely adhering E coli (DAEC) (Paton and Paton, 1998; Lisa and Rodgers, 1999; Burton and Paul, 2000; Prescott et al., 2003).

Fecal oral and food borne transmission of E coli have been well documented (Nataro and Kaper, 1998; Poppe et al., 2005). Epidemiologic studies have traced E coli out breaks to be of bovine origin and the strains express an unusual serotype, virulence factors and antibiotic resistance genes (Nataro and Kaper, 1998). Antimicrobial resistance in commensal strains of E coli has played an important role in the ecology of resistance and clinical infectious diseases (Winokur et al., 2001). Transmission of resistance genes from normally more virulent pathogenic species to nonpathogenic organisms is very common within the animal and human intestinal tract micro flora (Neidhardt, 1996; Winokur et al., 2001; Poppe et al., 2005). This may be an important mechanism for acquiring clinically significant antimicrobial resistant organisms (Neidhardt, 1996). E coli may serve as an important resistant antimicrobial reservoir for E coli or for some other pathogens. It is now clear that E coli has developed a number of elaborate mechanisms for acquiring and disseminating plasmids, transposons, phage and other genetic determinants (Neidhardt, 1996).

Plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera (Miranda et al., 2004). Unfortunately, there are still large gaps in our understanding of how new multi-resistance plasmids evolve. According to Armstrong et al., (1996), Smith et al., (2003) and Miranda et al., (2004) infections caused by E coli have become a significant public health problem world wide with the evolution of multi-resistance antibiotic plasmids genes. The work was carried out from 2001- 2004 to evaluate the utilization of ampicillin among diarrheagenic patients and also to determine the increasing Escherichia coli resistant to ampicillin in relation to their plasmids profile. The information obtained, will be used to augment the present knowledge on ampicillin resistance in our community by providing information on the appropriate choice of

antimicrobial agents. This definitely, will help in developing proper measures aimed at controlling ampicillin resistance bacteria; improve the quality of ampicillin prescription and usage of other antibiotics. This will also help to trace highly resistance isolates among enteric organisms in our environments where such trials are still at bud.

Materials and Methods:

From October 2001 to June 2004 a total 400 fecal samples were obtained from diarrheagenic patients attending University of Benin Teaching Hospital (UBTH), Ahmadu Bello University Teaching Hospital (ABUTH), University College Hospital (UCH), and Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nigeria.

Bacteriology: Samples obtained were inoculated aerobically on sterile blood agar, MacConkey agar, Cystine lactose electrolyte deficient agar, eosin methylene blue, nutrient agar and nutrient broth (Cheesbrough, 2000) at 37 0C for 24 hours. The colonies of each representative isolates were then characterized using standard bacteriological method according to Cowan and Steel (1974). Other tests included gram stain, pigment production, hemolysin production, motility indole, urea, citrate and hydrogen sulfide utilization, oxidase, and sugar fermentation were used to isolate the enteric gram negative bacteria. They were further sub culture on nutrient agar slants and stored at 40C for further analysis.

Antibiotics Susceptibility Testing: Susceptibility were determined both by overnight broth-micro-dilution and agar disk diffusion methods as recommended by Bauer et al.,(1996) and National Committee for Clinical Laboratory Standard (NCCLS) (1997) using Oxoid- Mueller Hinton agar. The following antibiotics were used to screen for the resistance of the isolates; ampicillin (AMP) 30mcg, gentamicin (CN) 10mcg, streptomycin (S) 30mcg, tetracycline (T) 30mcg, ciprofloxacin (CIP) 10mcg, Nalidixic acid (NA) 30mcg, erythromycin 30mcg (E), Norfloxacin (NB) 30mcg (Optun Laboratories Nig Ltd., Nigeria). The zones of inhibition were then measured and the results recorded as sensitive (s) or resistance ® base on World Health Organization Drug Information (1993) and National Committee for Clinical Laboratory Standard (NCCLS).

Conjugation and Plasmids profiles: Conjugation were performed using E coli strains obtained from Nigerian Institute for Medical Research (NIMR) as recipient as previously described by Olukoya and Oni, (1990). The donors and recipients-plasmid - free - rifampicin resistant strains were incubated both on Luria broth culture (Difco Laboratories, Detroit, MI) at 370C for 18 hours. The transconjugants were selected on nutrient agar (Nutrient agar-International Diagnostic Group UK) medium supplemented with ampicillin 30µg/ml and rifampicin 200µg/ml to inhibit the growth of the donor and recipient respectively. Curing was carried out according to Miller (1982) and Olukoya & Oni (1990). The transconjugants were re-streaked onto fresh selective nutrient plates and their identities were re-confirmed on the basis of their biochemical methods and their antibiotics resistance pattern. The Birnboim and Doly (1979) method was employed for screening plasmids (rapid alkaline extraction) of donors and transconjugants. The plasmids DNA were then electrophoresed on 0.8% agarose gel, stained with 7µl/g ethidium

3

bromide. The DNA was then photographed and viewed using UV transillumination. The molecular weights and distances were then determined using standard methods according to Meyers, et al., (1976), Kloos, et al., (1981) and Birnboim and Doly (1979) using standard DNA molecular weight marker II (0.12-23.1kbp) of bacteriophage lambda HindIII (2027, 2322, 4361, 6557, 9416 and 23130) Cat number 236250 (Roche Diagnostic GmbH).

Results:

Ampicillin Utilization: From 2001 to 2004, we carried out a survey on the behavour toward ampicillin utilization among some patients attending some Teaching Hospitals in Nigeria. From a total of 1243 questionnaires set to evaluate the behavior of ampicillin usage among some subjects attending some major Teaching Hospitals in Nigeria. The Teaching Hospitals were chosen base on geographical stratification, which include UBTH (South), NAUTH (East), ABUTH (North) and UCH (West) The result shows that 723(58.2%) volunteers responded to the questionnaires, where only 83(11.4%) consult a doctor for prescription, 397(54.9%) from drug retailers while 142(20.1%) self prescribe and 56(7.7%) prescription was base on the advice of friends and relatives as shown in Table 1. Also as concern the complete consumption level of the antibiotics if prescribed by a doctor only 229(31.7%) take full regimen of the antibiotics while 494(68.3%) stop the consumption of the regimen when the sign and symptoms disappear as shown in Table 1A and Table 1B. None of the above mentioned Teaching Hospitals had a surveillance method for evaluating the emerging of antibiotics resistant pathogens.

Resistance Due to Ampicillin: Out of a total of 249 diarrheagenic clinical isolates of E coli strains obtained in June 2001 to June 2004 from the 4 different Teaching Hospitals, 78 (31.3%) were obtained from UBTH, 41(16.5%) from ABUTH, 38(15.3%) from NAUTH and 92(36.9%) from UCH as shown in Table 2 with a mean average of 63(25.3%). Table 2 also showed the varied antibiotics susceptibility pattern where ampicillin was the least susceptible to most of the E coli strains screened, followed by tetracycline. The MIC for ampicillin was ≥4μg/l for 98% of the ampicillin-resistant organisms tested. The most susceptible antibiotics to the E coli strains were ciprofloxacin and norfloxacin during the entire period of the study. Most of the strains screened for had one or more resistant R plasmids as shown in Table 3. When screened for Ampr, 52% of E coli isolates harbour plasmids of different molecular weights that ranged from ≤0.451kbp to ≥1.254kbp. The results also showed that mostly Chromosomal DNA mediated E coli isolates resistant to tetracycline, erythromycin, and ciprofloxacin.

Table 1 A: Ampicillin usage from some patients attending Teaching Hospital in Nigeria from 2001 to 2004.

Respondent	Total	Rarely	Frequently	Doctors prescription	Drug retailer Prescription	Pharmacist Prescription	Friends and relatives	Self Prescription	Full Regimen	Incomplete Regimen
House wife	143	132(92.3%)	11(7.7%)	10(7.0%)	82(5.7. %)	6(4.2%)	13(9.1%)	32(22.4%)	16(11.2%)	127(88.8%)
Drivers	90	83(92.3%)	7(7.8%)	6(6.7%)	53(58.9%)	2(2.2%)	7(7.8%)	22(24.4%)	13(14.4%0	77(85.6%)
Students	200	179(89.5%)	21(10.5%)	13(6.5%)	97(48.5%)	8(4.0%)	16(8.0%)	66(33.0%)	51(25.5%)	149(74.5%)
Teachers	80	71(88.7%)	10(11.3%)	32(40.0%)	29(36.3%)	13(16.3%)	5(6.3%)	1(1.3%)	38(47.5%)	42(52.5%)
Lawyers	26	24(92.3%)	2(7.7%)	15(57.7%)	7(26.9%)	3(11.6%)	-	1((3.9%)	19(73.1%)	7(26.9%)
Trader	123	119(96.7%)	4(3.3%)	3(2.4%)	95(77.3%)	2(1.6%)	5(4.1%)	18(14.6%)	79(64.2%)	44(35.8%)
Others	61	54(88.5%)	7(11.5%)	3(4.9%)	34(55.7%)	8(13.1%)	10(16.4%)	6(9.8%)	13(21.3%)	48(78.7%)
Total	723	662(91.6%)	62(8.6%)	83(11.5%)	397(54.9%)	42(5.8%)	56(7.7%)	145(20.1%)	229(31.7%)	494(68.3%)

Table 1B: Ampicillin usage from some patients attending Teaching Hospital in Nigeria from 2001 to 2004.

Typhoid	Diarrhea	Sore throat	Body pain	No reason
70	61	11	80	13
32	58	14	61	15
11	19	-	-	-
7	3	1	4	-
2	4	-	5	-
2	39	-	82	-
13	28	-	32	5
137	212	26	264	33

Table 2: Antibiotic Susceptibility Pattern of E coli Isolated from some Teaching Hospital in Nigeria from 2001 to 2004

Teaching Hospital	Source of Sample	Total No of E coli Isolates	AMP	CIP	CN	S	E	T	NB	NA
UBTH	Faeces	78(31.3%)	6(7.7%)	67(85.9%)	23(29.5%)	22(28.2%)	13(16.7)	38(48.7%)	68(87.2)	48(61.5%)
NAUTH	Faeces	38(15.3%)	15(39.5%)	31(81.6%)	14(36.8%)	10(26.3%)	9(23.7%)	11(28.9%)	28(73.7%)	19(50.0%)
ABUTH	Faeces	41(16.5%)	11(26.8%)	36(87.8%)	19(46.3%)	17(41.5%)	18(43.9)	5(12.3%)	25(61.0%)	23(56.1%)
UCH	Faeces	92(36.9%)	9(9.8%)	78(84.8%)	32(34.8%)	21(22.8%)	13(14.1%)	34(37.0%)	82(89.1%)	46(50.0%

Key: AMP: Ampicillin, CIP: Ciprofloxacin, CN: Gentamicin, S:Streptomycin, T: Tetracycline, NB: Norfloxacin, NA: Nalidixic acid, E: Erythromycin

Table 3: Antibiotics Resistance Patterns and Plasmids Profiles of *Escherichia coli* from 2001 to 2004 in Nigeria

Isolates	Antibiotics	No with Plasmids	Plasmids size (kbp)	Transferred Plasmids Size (kbp)	Frequency of Transfer
A022	NaAmpNbCipTECn	3	1.25, 0.87, 0.451	1.25, 0.87	6×10^{-4}
A029	EAmpNbCn	2	1.06, 0.77	1.06, 0.77	3×10^{-4}
A030	NbNaT	0	-	-	-
A316	NbCnAmpCipET	2	1.25, 0.87	1.25	6×10^{-2}
A032	NaAmpGnT	2	1.25, 0.87	1.25	6×10^{-2}
A401	NaAmpTNbT	2	1.06, 0.77	1.06, 0.77	3×10^{-1}
A043	NbECn	0	-	-	-
U02	Amp	1	1.25	1.25	6×10^{-2}
U09	CnNbTNaAmp	2	1.25, 0.87	0.87	6×10^{-2}
U15	Amp	1	1.06	1.06	6×10^{-2}
U18	NbCnNaAmpT	2	1.06, 0.77	1.06	3×10^{-4}
U201	NbCnTENaAmpCip	3	1.06, 0.77, 0.451	0.77, 0.451	6×10^{-2}
N01	CnAmpCipNa `	3	1.25, 1.06, 0.87	1.25, 0.87	6×10^{-2}

Key: AMP: Ampicillin, CIP: Ciprofloxacin, CN: Gentamicin. S:Streptomycin, T: Tetracycline, NB: Norfloxacin, NA: Nalidixic acid, E: Erythromycin
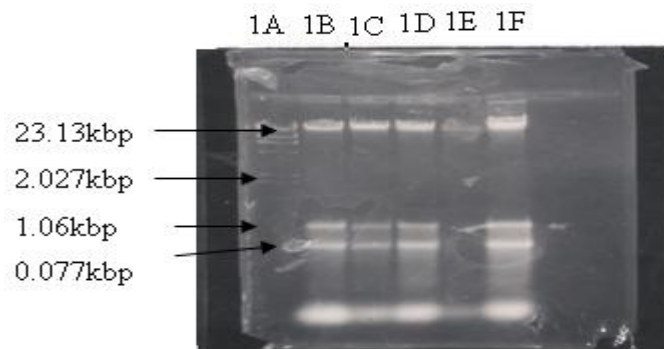

Fig1: Plasmids DNA of diarrheagenic clinical isolates of E coli from 4 different Teaching Hospitals in Nigeria. Separation of DNA molecular weight on agarose gel stained with ethidium bromide. Line 1A = Standard bacteriophage Lambda DNA fragment, Lines 1B, 1C, 1D, 1E and 1F are DNA fragments of test isolates.
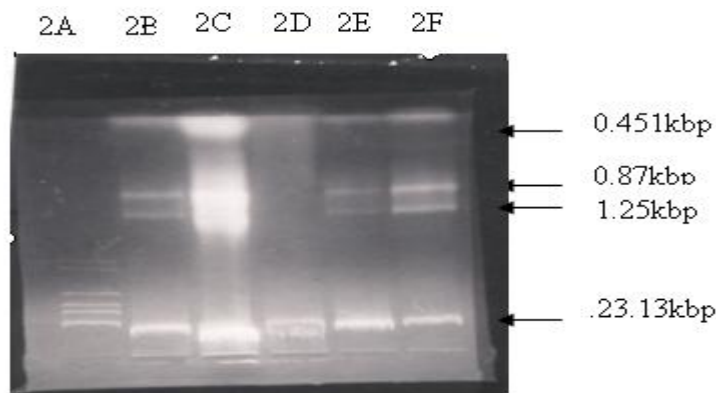

Fig2: Plasmids DNA of diarrheagenic clinical isolates of E coli from 4 different Teaching Hospitals in Nigeria. Separation of DNA molecular weight on agarose gel stained with ethidium bromide. Line 2A = Standard bacteriophage Lambda DNA fragment, Lines 2B, 2C, 2D, 2E and 2F are DNA fragments of test isolates.

6

Discussion:

Multiple antibiotics resistance to useful classes of antibiotics including beta lactams, aminoglycosides and quinolones has generally increased among a number of gram-negative hospital pathogens. The driving force of the antibiotic resistance being the widespread use of antimicrobial drugs as indicated from the ampicillin usage. In the study 145(20.1%) of the population self prescribe, 56 (7.7%) prescription due to friends and relatives, 397(54.9%) from drug retailers, 42(5.8%) from pharmacists and 83(11.3%) from doctors. Among those who adhere to doctor's prescription only 229(31.7%) take full ampicillin regimen when the signs and symptoms of the infection disappear while 494(68.3%) discontinue the regimen when the signs and symptoms subside.

In developing countries, diarrhea may be infective and non-infective but the fact remains that most of them are self-limiting and require only adequate rehydration. Acquired diarrhea bacterial resistance to various antimicrobial agents is common in isolates from both healthy persons and from persons with community-acquired infections (Okeke et al., 1999) most of them driven by complex socioeconomic and behavioral factors associated with antibiotic resistance especially in diarrheal pathogens (Okeke et al., 1999; Olukoya & Oni 1990). These include misuse of antibiotics by health professionals, unskilled practitioners, and laypersons; poor drug quality; unhygienic conditions accounting for spread of resistant bacteria; and inadequate surveillance (Goldman, et al.1996; Weinstein, 2001; Okeke et al., 1999).

Among isolates of diarrheal commensal enteric pathogens (Murray et al., 1985; Sack et al., 1997; Rahal et al., 1997; Hoge et al., 1992), resistance is increasing, particularly to first-line, inexpensive, broad-spectrum ampicillin and others. This account for the fact that most health workers in many developing countries have almost no access to objective health information (Lee and Henry, 1989) and well-trained health personnel are scarce and cannot serve the entire population, especially in rural areas (Okeke et al., 1999). These unskilled personnel are less aware of the deleterious effects of inappropriate antibiotic use. These unqualified drug sellers offer alternative drugs when the prescribed drugs are out of stock or refill prescriptions without consulting the prescriber (Singh and Raje, 1996; Haak, 1988). In most developing countries, ampicillin can be purchased without prescription, even when the practice is not legal. In these less developed countries, ampicillin is readily available on demand from hospitals, pharmacies, patent medicine stalls (drugstores), roadside stalls, and bus hawkers (Nizami et al.,1996; Haak, 1988; Fagbule and Kalu, 1995; Obaseki et al.,1987; Lansang et al.,1990; Okeke, 1995; Hossain et al., 1982; Goel et al,1996). In rural Bangladesh, for example, 95% of drugs consumed for 1 month by more than 2,000 study participants came from local pharmacies; only 8% were prescribed by physicians (Calva et al., 1993). People are encouraged to buy from unofficial distributors because drugs often are not available in government hospitals (Abosede, 1984). Drug vendors usually have little or no knowledge of the required dosage regimen, indications, or contraindications are the one in charge of the dispensing of drugs (Haak, 1988; Rahman et al., 1998;Abosede, 1984). In markets, long distance buses-public transport in Nigeria the vendor (untrained salesman) tries to convince potential buyers

to purchase the drug, even if they are not ill (Okeke et al., 1999). To save time and keep drug-hunting to a minimum, a patient may start at a source more likely to stock the desired drug, forgoing the expertise of a doctor. As shown from the study, most patients prefer the advice of drug retailers because their sales depends on the amount the patient have not on the dosage to inhibit the pathogen.

Unofficial sources are generally more accessible than official sources. In Nigeria for example, retail drug outlets are more numerous than government health posts and hospitals. Alternate sources offer the option of purchasing small quantities of ampicillin, while hospitals require purchase of the complete 5- or 7-day ampicillin regimen (Nizami et al., 1996; Haak, 1988; Hossain et al., 1982). Also as shown from the study 54.1% of the masses in Nigeria prefer the advice of drug retailers for their health problems while just 11.5% seek the opinion of a doctor for their health problems. In some localities, these unofficial sources especially in the villages they called doctors.

The purchase of small samples is exceedingly common, particularly for most customers, who buy without prescription (Hossain et al., 1982). These sub inhibitory ampicillin regimens predispose for selection of resistant bacterial strains of E coli and other pathogens. Ampicillin use in developing countries is underestimated. However, medication can be purchased in small aliquots from roadside stalls, and distribution of locally produced or counterfeit antibiotics is not recorded. Government and other health agencies should highlights the control system as well as monitor and regulate the use/distribution of antibiotics as the case with

National Agency for Food, Drug and Administrative Control (NAFDAC). This reduces the counterfeit level and will also help reduce low quality standard antibiotics supply in our health system.

The motives for self- medication and ampicillin overuse by laypersons are similar to those for clinical abuse by health professionals: to cut costs and to treat confirmed or suspected bacterial infection (Yang et al., 1993). For example, 50% to 80% of Bangladeshi patients infected with Shigella admitted that they had taken at least one antibiotic in the 15 days before a hospital visit (Catalamo et al., 1990), as had 18% to 70% of pediatric patients with acute respiratory infection in two Chinese studies (Reyes et al., 1997; Kuin et al., 1987). The proportion of patients who self-medicate is probably higher, because patients are often reluctant to admit having taken antibiotics before visiting a hospital (Haak and Hardon, 1988). This confirms the recent study where 20.1% self medicate and only 11.5% seek the advice of a physician for their health problems.

Common cultural beliefs about antibiotics include the notions that there is a pill for every symptom; ampicillin can heal many illnesses. The misuse of ampicillin and other antibiotics frequently becomes integrated into the local culture; ampicillin used to prevent diarrhea after eating suspected contaminated foods can also be used to prevent sexually transmitted diseases, wound infections, ear infections (Hossain et al.,1982; Agom et al., 1990; Strang, 1996; Abellanosa&Nichter, 1996; Yah et al.,2004; Esezobo and Offiong, 1986). Another cause of ampicillin abuse and selection for resistant bacteria is poor patient compliance. First, physician-patient

interactions are often inadequate. Since health cares are scarce, patients often travel long distances and incur large expenses for medical care; they are unlikely to return for follow-up visits (Okeke et al., 1999). Also as observed from the study, 68.3% undergo incomplete regimen as compared to 31.7% who do take full complete regimen. This is because many antibiotics are expensive, indigent patients purchase incomplete regimens whenever possible and discontinue treatment when symptoms disappear allowing the pathogen at sub lethal stage encouraging fast emerging resistant pathogens.

Ampicillin resistance from the study was highly mediated by plasmids although chromosomal mutations have been observed in other studies. The gene responsible for plasmids mediated resistance ampr was found in plasmids varying in size from ≤0.451kbp to ≥1.254kbp in clinical isolates of E coli strains. It was confirmed by the in-vitro results on transconjugants, which were selected with Rifampicin 200≥µl/g at a conjugation frequency of 3X10-4 to 6X10-4. The E coli ampicillin resistance strains were found in 85% to 100% of rifampicin resistance strains of transconjugants tested. The ampr positive plasmids in transconjugants were the same size as those in their respective donors. Transfer was not successful in all the strains, although positive during curing Procedures. The plasmids or the genetic capability may have been lost during repeated successive sub-cultures of the isolates. The fact that ampr are plasmids borne is supported by plasmids pMG252, the first plasmids found to carry AMPC – type-beta lactamase FOX-5 which have found to encode qnr (quinolones) resistance (Minggui et al., 2004). The work had a high diversity of E coli plasmids resistance to ampicillin as

against the work of Olukoya and Oni, (1990) who found that ampicillin resistant Shigella in Nigeria were mostly chromosomally borne rather than plasmids borne. Although Shigella and E coli are members of the enteric family and genetic transfer is very common.

The study also revealed that there is no consistent relationship between E coli ampicillin resistant pattern and the number of plasmids bands present. 3 of the isolates were found resistant to more than 5 antibiotics and had plasmids while another only one plasmid. Probably the ampicillin resistance in these isolates which did not posses plasmids were chromosomally and or transposons borne. There was also a high degree of plasmids relatedness among the E coli isolates from the various Teaching Hospitals because of the presence of similar size plasmids especially 0.77, 0.87, 1.06 and 1.25kbp. According to Nashwan, et al.,(2005)and Heritage, et al.,(2003) the transfer of resistance genes between different bacterial species may go unnoticed by traditional infection control and epidemiological methods, thereby undermining hospital infection control polices. Nashwan,et al., (2005) concluded that plasmids outbreaks may go unrecognized but also that stringent measures directed against all multi-drug resistance gram negative bacilli can be effective in controlling outbreaks if proper hygiene, wearing of gloves and gowns during patient care are observed. Finally the presence of plasmid DNA in some E coli ampicillin resistant strains demonstrated that resistance was plasmid mediated and this could have resulted from the abuse/misuse or other selected pressures sufficient for the broad geographic distribution of ampr genetic linkage.

References:

1- Burton, GRW, Paul GE . Medical Microbiology for Health Science. 6th Ed., Linppincott Williams & Wilkins.2000. Pp 402-403

2- Prescott ML, Harley JP, Klein DA. Microbiology, 4th Edit. The Tata McGraw Hill:1999. 792-802.

3- Smith SI, Aboaba OO, Odeigha P, Shodyo K, Adeyeye JA, Ibrahim A, Adebiyi T, Onibokum H, Odunukwe NN. Plasmids profiles E coli from apparently healthy animals. African journal of Biotechnology (AJB).2003.Vol 2(9): 322-324.

4- Lisa AS, and Rodgers AI. Essentials of diagnostic microbiology. Delmar publisher's. 1999 pp 186-205.

5- Armstrong GL, Hollingsworth J, Morris JG. Emerging foodborne pathogens. Escherichia coli 0157:H7 as a model of entry of a new pathogen into the food supply of developed world. Epidemiol Rev.1996.Vol 18: 29-51.

6- Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AMPc- lactamase plasmids between E coli and Salmonella isolates food, animals and humans. Antimicrobial agents & chemotherapy.2001.Vol 45(10): 2716-2722.

7- Nataro JP, Kaper JB. Diarrheagenic Escherichia coli Clin. Microbiol. Rev.1998, Vol 112: 142-201.

8- Neidhardt FC. Escherichia coli and Salmonella. Cellular and molecular biology. ASM. Press, Washington, D.C.1996.

9- Poppe C, Martin LC, Gyles CL, Reid-Smith R, Boerlin P, McEwen SA, Prescott JF, Forward KR. Acquisition of resistance to extended spectrum cephalosporin by Salmonella enterica subsp. Enterica serovar Newport and Escherichia coli in Turkey Poult Intestinal Tract. Appl. Environ. Microbiol 2005 Vol 71: 1184- 1192.

10- Sayah RS, Kaneene JB, Johnson Y, Miller R. Patterns of antimicrobial resistance observed in Escherichia coli isolates obtained from domestic and wild animals fecal samples, human septage and surface water. Appl. Environ. Microbiol 2005 Vol 71: 1394- 1404.

11- Miranda S, David MG, Peter JC. (2004). Evolution of multi- resistance plasmids in Australia clinical isolates of Escherichia coli. .Microbiology.2004.Vol 150: 1539-1546.

12- Cheesbrough M. District Laboratory Practice Manual in Tropical Countries. Part 2. Cambridge University Press. 2000. Pp 178-179.

13- Cowan ST, Steel KJ. Manual for the identification of medical bacteriology. Cambridge University Press. London, New York, Rockville, Melbourne and Sydney.1974

14- National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility test for bacterial that grow aerobically. 4th edition. Approved Standards M7-A4 NCCLS, Villanova, P.A 1997.

15- World Drug Information. Essential Drugs Guidelines for antimicrobial susceptibility testing. 1993. Vol 7: 68-78.

16- Bauer AW, Kirby WM, Sherris JC. Antibiotics susceptibility testing by a standardized single disk method. Am.J. Clin. Patho 1996 Vol. 45: 493-496.

17- Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant DNA. Nucleic acids Res 1979 Vol 7: 1513-1523.

18- Olukoya DK, Oni O. Plasmid profile analysis and antibiotics susceptibility patterns of Shigella isolates from Nigeria. Epidemiol. Infect.1990 Vol 105: 59-64.

19- Miller, H.J.H. Experiment in molecular genetics cold spring hanbor New York.1982

20- Meyers JA, sanchez D, Elwell LP, Falkows. Simple agarose gel electrophoretic method for the identification and characterization of plasmids deoxyribonuclease acid. J.Bacterial 1976 Vol 127: 1529-1537.

21- Kloos WE, Oraban BS, walker DD. Plasmids composition of Staphylococcus species. Can. J. microbial 1981 Vol 27: 271-278.

22- Paton JC, Paton AW. Pathogenesis and diagnosis of shiga-toxin producing Escherichia coli infections. Clinical Microbiology Reviews 1998 Vol 11, 450-479.

23- Goldman DA, Weintein RA, Wenkel RP, Talban OC, DAma RJ, Gaynes RP. (Strategies to prevent and control the emergence and spread of antimicrobial –resistance microorganisms in hospital: A Challenge to hospital leadership. JAMA 1996 Vol 275: 234-40.

24- Weinstein RA. Controlling antimicrobial resistance in Hospitals: Infection control and use of antibiotics. Special issue. 2001 Vol 2 :1-10.

25- Okeke I N, Adebayo L, Robert E. Socioeconomic and Behavioral Factors Leading to Acquired Bacterial Resistance to Antibiotics in Developing Countries. URL:http://www.cdc.   gov/ncidod/EID/vol5no1/okeke.htm , 1999 Vol 10:25:01

26- Murray BE, Alvarado T, Kim KH, Vorachit M, Jayanetra P, Levine MM Increasing resistance to trimethoprim-sulfamethoxazole among isolates of Escherichia coli in developing countries. J Infect Dis 1985 Vol 147:724-8.

27- Sack RB, Rahman M, Yunus M, Khan EH. Antimicrobial resistance in organisms causing diarrheal disease. Clin Infect Dis 1997. Vol 24 Suppl 1:S102-5.

28- Rahal K, Wang F, Schindler J, Rowe B, Cookson B, Huovinen P,et al., Reports on surveillance of antimicrobial resistance in individual countries. Clin Infect Dis 1997 Vol 24 Suppl 1:S169-75.

29- Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin Infect Dis 1998 Vol 26:341-5.

30- Williams RJ, Heymann DL. Containment of antibiotic resistance . Science. (1998) Vol 279:1153-4.

31- Singh J, Raje N. The rise of Western medicine in India. Lancet (1996). Vol 1348:1598.

32- Haak HV. Pharmaceuticals in two Brazilian villages: lay practices and perceptions. Soc Sci Med . (1988). Vol 27:1415-27.

33- Nizami SQ, Khan IA, Bhutta ZA. Drug prescribing practices of general practitioners and paediatricians for childhood diarrhoea in Karachi, Pakistan. Soc Sci Med 1996 Vol 42:1133-9.

34- Fagbule D, Kalu A. Case management by community health workers of children with acute respiratory infections: implications for national ARI control programme. J Trop Med Hyg . 1995 Vol 98:241-6.

35- Obaseiki-Ebor EE, Akerele JO, Ebea POA survey of antibiotic outpatient prescribing and antibiotic self- medication. J Antimicrob Chemother .1987 Vol 20:759-63.

36- Lansang MA, Lucas-Aquino R, Tupasi TE, Mina VS, Salazar LS, Joban N, et al.: Purchase of antibiotics without prescription in Manila, the Philippines. Inappropriate choices and doses. J Clin Epidemiol 1990 Vol 43:61-7.

37- Okeke I, Lamikanra A.. Quality and bioavailability of tetracycline capsules in a Nigerian semi-urban community. Intern. J Antimicrobial Agents.1995 Vol 5:245-50.

38- Hossain MM, Glass RI, Khan MR. Antibiotic use in a rural community in Bangladesh. Int J Epidemiol.1982 Vol 11:402-5.

39- Goel P, Ross-Degnan D, Berman P, Soumerai S. Retail pharmacies in developing countries: a behavior and intervention framework. Soc Sci Med 1996 Vol 42:1155-61.

40- Calva JJ, Ceron E, Bojalil R, Holbrook A. Antibiotic consumption in a community of Mexico City. II. Survey of purchases at pharmacies. Bol Med Hosp Infant Mex.. 1993 Vol 50:145-50

41- Rahman F, Andersson R, Svanstrom L. Medical help seeking behaviour of injury patients in a community in Bangladesh. Public Health . 1998 Vol 112:31-5.

42- Fagbule D, Kalu A. Case management by community health workers of children with acute respiratory infections: implications for national ARI control programme. J Trop Med Hyg. 1995 Vol 98:241-6.

43- Okeke I, Lamikanra A. Quality and bioavailability of tetracycline capsules in a Nigerian semi-urban community. Int J Antimicrobial Agents . 1995 Vol 5:245-50.

44- Hossain MM, Glass RI, Khan MR. Antibiotic use in a rural community in Bangladesh. Int J Epidemiol. 1982 Vol 11:402-5.

45- Abosede OA. Self-medication: an important aspect of primary health care. Soc Sci Med. 1984 Vol 19:699-703.

46- Shahid NS, Rahaman MM, Haider K, Banu H, Rahman N. Changing pattern of resistant Shiga bacillus (Shigella dysenteriae type 1) and Shigella flexneri in Bangladesh. J Infect Dis. 1985 Vol 152:1114-9.

47- Yang YH, Fu SG, Peng H, Shen AD, Yue SJ, Go YF, et al. Abuse of antibiotics in China and its potential interference in determining the etiology of pediatric bacterial diseases. Pediatr Infect Dis J. 1993 Vol 12:986-8.

48- Catalano M, Almiron MA, Romeo AM, Caruso E, Murtagh P, Harisiadi J. Comparison between parental report and results of microbiologic agar assay for presence of antibiotic in urine of Argentinian children with acute lower respiratory tract infection. Reviews of Infectious Diseases .1990 Vol 12 Suppln 8:S998- 1000.

49- Kunin CM, Lipton HL, Tupasi T, et al. Social, behavioral, and practical factors affecting antibiotic use worldwide: report of Task Force 4. Reviews of Infectious Diseases.1987 Vol 9 Suppl 3:S270-S85.

50- Reyes H, Guiscafre H, Munoz O, Perez-Cuevas R, Martinez H, Gutierrez G. Antibiotic noncompliance and waste in upper respiratory infections and acute diarrhea. J Clin Epidemiol. 1997 Vol 50:1297-304.

51- Yah SC, Enabulele IO, Eghafona NO.

Bacteriological studies of infected kerosine burn

wounds in Benin City, Nigeria.

JBI 2004 Vol 2(1): 4-9.

52- Strang JK. Tracing patients in rural Africa. Lancet.1996 Vol 348:1083-4.

53- Esezobo E, Offiong E. In vitro studies on some brands of oxytetracycline capsules available in Nigeria. Nig J. Pharmacology. 1986 Vol 17:24-8.

54- Agom JK, Akanni AO, Dawodu TO. Quality of ampicillin/cloxacillin   preparations on the Nigerian market. Nig J Pharmacology . 1990 Vol 21:36-8.

55- Haak H, Hardon AP. Indigenised pharmaceuticals in developing countries: widely used, widely neglected. Lancet 1988;2:620-1

56- Minggui W, Daniel FS, George AJ, David C H. Emerging plasmid mediated quinolones resistance associuated with the qnr gene in Klebsiella pneumoniae clinical isolates in the United States. Antimicrob Agents Chemother 2004 Vol 48(4) 1295-1299.

57- Nashwan, A N., Birgitta, D., Paul, H.M.S.,Lodewijik, S.., Evert de J., Aldeert, B., Christina, M.V.G. and Menno, D de J. Widespread transfer of Resistance Genes between Bacterial Species in an Intensive Care Unit: Implementations for Hospital Epidemiology. Journal of Clinical Microbiology 2005 Vol 43(9): 4862-4864.

58- Heritage, J., Chambers, P.M., Tyndall, C. and Buescher, E.S. SHV-34 an extended-spectrum beta lactamase conferring transferable resistance encoded by an epidemic plasmid. J. Antimcrob. Chemother 2003. 52: 1015- 1017.