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Full Length Research paper

Transfer of gentamicin resistance genes among enterobacteriaceae isolated from the outpatients with urinary tract infections attending 3 hospitals in Mubi, Adamawa State

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300 urine samples with significant bacteriuria collected from 3 hospitals in Mubi were analysed for presence of Enterobacteriaceae bacteria. 187 urine samples comprising 68.9% female and 31.1% male yielded Enterobacteriaceae bacteria growth. The isolates include *Escherichia coli* 51.5%, *Klebsiella pneumonia* 24.4%, *Klebsiella oxytoca* 3.1%, *Enterobacter aerogenes* 9.7% and *Citrobacter freundii* 10.9%. Antibiotic resistance profile obtained revealed high resistance of isolates to ampicillin 37.5%, ciprofloxacin 36.4% and co-amoxyclav 21.3%. Streptomycin, nalidixic acid, cephalexin and gentamicin highly inhibited growth of the organisms tested. Gentamicin resistance rate of 17% was obtained while curing of selected donor isolates showed that gentamicin resistance in 75.8% of the isolates were plasmid mediated or located on mobile genetic element. Transfer rates of 34.8% and 41.1% respectively were obtained for inter-generic and intra-species transfer of gentamicin resistance genes (Gm^r) among the Enterobacteriaceae isolates. Evidence of transferability of Gm^r *in vitro* concurs to the assertion that under favorable conditions conjugal transfer of gentamicin resistance determinants and hence R plasmid could occur *in vivo*.

Key words: Enterobacteriaceae, gene transfer, gentamicin resistance genes, plasmid, curing, conjugation.

INTRODUCTION

The introduction of antimicrobial drugs most notably penicillin was thought to herald the beginning of the end of bacterial infections. Unfortunately the rapid recognition of penicillin resistance within a year of its introduction disabused physician of this notion (Furuya and Lowy, 2005). Multiple antibiotic resistance to useful classes of antibiotics including the beta-lactams, aminoglycosides and quinolones has generally emerged and this has been increasingly observed among a number of gram-negative pathogens such as the Enterobacteriaceae bacteria (Yah et al., 2006). Initially infections caused by antimicrobial resistant bacteria occurred mainly in hospital settings, where antibiotic use was most extensive. Thus bacteria carrying antimicrobial resistance genes had survival advantage that facilitated dissemination in this setting (Dzidic and Bedekovic, 2003). However, Furuya and Lowy (2005) observed a disturbing trend on the spread of antimicrobial resistant bacteria within the community. The foremost reason for this trend is the increasing volumes of antimicrobial usage and studies indicate correlation between usage and the extent of antimicrobial resistance (Goossens et al., 2005).

Antibiotic resistance in bacteria develops either by mutation or acquisition of new genes through a process known as horizontal gene transfer. This involves the transfer of resistance genes among pathogens which are often facilitated by the localization of these genes on plasmids, particularly those associated with integrons and transposons (Tenover, 2006). Numerous studies have shown that resistance genes could be transferred from one bacterium to the other both *in vitro* and *in vivo*. Transfer of resistance genes between *Shigella* sp. and *Escherichia coli* has been observed (Hooper, 2000; Tenover, 2006). Aluyi and Akortha (2002) reported *in*

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vitro intra- and inter-species gene transfer of ampicillin resistance genes among enteric bacteria of diarrhea origin. Shoemaker et al. (2001) observed an extensive transfer of tetracycline resistant determinants among bacteroids and other enteric bacteria in the human colon. In the same vein, transfer of resistance and virulence genes was demonstrated between *Salmonella* sp. inside epithelial cells. This dissemination of antibiotic resistance genes has led to rapid emergence and spread of antibiotic resistance among bacteria populations and thus pathogens involved in urinary tract infections.

Gentamicin resistance genes aac (3)-IIa and aac (3) – VIa have been reportedly detected in gram-negative bacteria in the clinical settings. Sequencing and PCR experiments have confirmed that these genes are present on mobile genetic element that can facilitate their horizontal transfer among bacteria (Diaz et al., 2006).

As the proliferation of multidrug resistance pathogens continue unavoidably within and around us, it is important that their resistance trend be put under check through intensive research and antibiotic surveillance. The bacteria of the Enterobacteriaceae family have emerged as major pathogens of interest in part because of their resistance to multiple antibiotics which confers on it survival advantage. With its propensity to acquire such traits, as resistance determinants to various antimicrobial drugs and other virulence factors, the Enterobacteriaceae will continue to create new therapeutic problems and dilemmas. Their ability to transfer plasmids to other species and genera of bacteria are of great concern. In the light of this, a study on the implication of Enterobacteriaceae bacteria in urinary tract infections (UTI) was carried out in Mubi with a view to determine its prevalence and antimicrobial resistance profile to various antibiotic, but with specific interest on gentamicin, an antibiotic sold in ampule hence believed not to be abused as those sold in capsules and tablets. Attempt will be made to demonstrate *in vitro* transferability of gentamicin resistance determinants among replicons of the Entero-bacteriaceae isolates.

MATERIALS AND METHODS

Study area

The study area is Mubi, a town comprised of Mubi South and Mubi North Local Government area of Adamawa State. Mubi is located in North-East region of Nigeria, between latitudes 10° 1'N and 10° 20'N longitudes 13° 1'E and 13° 30°E. It occupies a land area of about 725.85 Km^2 with an estimated population of about 300,000 people (Adebayo, 2004; Federal republic of Nigeria official gazette, 2007). Mubi consists of a general hospital that serves as a referral center to other private and public health institutions around, 3 state owned tertiary institutions and a Federal Polytechnic.

Sample collection

About 386 urine samples were collected using sterile universal bottle from outpatients attending Specialist hospital, New Life Hospital and Beekey Hospital in Mubi. Information on age and sex were appropriately recorded and the samples were further analyzed for significant bacteriuria and the presence of the Enterobacteriaceae bacteria.

Isolation and characterization of Enterobacteriaceae bacteria Isolates

Using a standard wire loop, a loopfull of urine sample which is equivalent to 1/250 ml was transferred on to nutrient agar plate (Antec) and spread using bent glass rod. After incubation at 37°C for 24 h, plates with bacteria count of 10^5 were taken as significant for bacteriuria (Baker and Silverton, 1985; Wright et al., 1999). The urine samples were also plated on MacConkey agar (Biochemika) and CLED agar (Sigma) using sterile wire loop and incubated at 37°C for 24 h. The isolates were sub-cultured on to nutrient agar, gram stained and subjected to the following biochemical tests, oxidase, catalase, citrate, indole, motility, urease, hydrogen sulfide and lactose (Koneman et al., 1994; Chesbrough, 2002). Identified cultures were preserved on nutrient agar slant for further analysis.

Susceptibility test of isolates to antibiotic

Antibiotic susceptibility test was performed using the standardized inoculum disk diffusion test as described by Kirby and Beuer (1966) and NCCL (1998). The antibiotic disk (optudisc) used contained the following antibiotics: tarivid (oflaxacin) 10 µg; perflaxin (perfloxacin) 10 µg; ciprofloxacin (ciprolonic) 10 µg; co-amoxycla (amoxicillin - cluvalanic acid) 30 µg; nalidixic acid 30 µg; ciporex (cephalexin) 10 µg; gentamicin 10 µg; streptomycin 30 µg; co-trimexazole (trimethoprim-sulphamethoxazole) 30 µg and ampicillin 30 µg.

5 colonies of each isolate were picked and transferred into 5 ml of sterile nutrient broth. The broth was incubated at 37°C for 5 h. The turbidity of the suspension was adjusted with sterile broth to match the turbidity standard of Mcfarland solution.

The suspension was mixed properly to evenly spread the colonies. A sterile swab stick was dipped into the suspension, excess fluid was removed before evenly spreading the colonies on Mueller Hinton agar (MHA; Biomark™) and allowed to dry for 5 - 10 min before the antibiotic disk were applied using sterile forceps. The plates were incubated at 35°C for 18 h. After incubation, the criteria of NCCL (1998) were used to interpret the zone sizes of each antimicrobial agent.

Curing of donor and recipient isolates

Drug resistant isolates which were resistant to gentamicin and sensitive to nalidixic acid were used as donor, while those resistant to nalidixic acid but sensitive to gentamicin which were used as recipients.

Sub-inhibitory concentration of 0.10 mg/ml of acridine orange was used for plasmid curing (Silhavy et al., 1984). The selected isolates were grown for 24 h at 37°C in nutrient broth containing 0.10 mg/ml acridine orange. After 24 h, the broth was agitated to homogenize the content and loopfull of the broth medium was sub cultured on to MHA plates. The plates were incubated at 37°C for24 h after which colonies were screened for antibiotic resistance by the disk diffusion method. This was to confirm whether the resistance was borne on plasmid or on the chromosome.

Determination of minimum inhibitory concentrations of gentamicin and nalidixic acid

Minimum inhibitory concentrations of gentamicin and nalidixic acid

Table 1. Distribution of the Enterobacteriaceae bacteria by sex.

Bacteria isolate	Male	Female	Total
E. coli	36(36.4%)	62(62.6%)	98 (51.5%)
K. pneumoniae	9(19.2%)	38(80.8%)	47 (24.4%)
K. oxytoca	2(33.3%)	4(66.7%)	6 (03.1%)
E. aerogenes	4(21.1%)	15(78.9%)	19 (09.9%)
C. freundii	7(33.3%)	14(66.6%)	21 (10.9%)
Total	58(30.2%)	133(69.3%)	191(100%)

Table 2. Frequency of isolates resistant to selected antimicrobial agents.

Antibiotics	Bacteria isolates					Total
	E. coli	*K. pneumoniae*	*K. oxytoca*	*E. aerogenes*	*C. freundii*	
Pn	39	18	2	6	7	72 (37.5%)
Ofx	21	7	2	5	3	38 (19.8%)
Nal	13	6	3	6	1	29 (15.1%)
Pef	16	11	2	6	1	37 (19.3%)
Gm	17	9	1	3	3	33 (17.7%)
Au	23	13	0	3	2	41 (21.3%)
Cpx	35	20	2	7	6	70 (36.4%)
Sxt	14	9	2	7	3	36 (18.8%)
Cep	21	3	0	3	4	31 (16.2%)
S	14	5	1	2	3	25 (13.0%)

Abbreviations: Au - Amoxicillin-clavulanic acid, Nal - Nalidixic acid, Pn - Ampicillin, Cep - Cephalexin, Ofx - Ofloxacin, S – Streptomycin Cpx - Ciprofloxacin, Pef - Perfloxacin, Sxt - Trimethoprim - sulphamethozole, Gm - Gentamicin.

were determined using agar dilution method (EUCAST, 2000; Andrew, 2001). MHA supplemented with various concentration of gentamicin; 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 µg/ml were prepared. To each set of MHA containing the various concentrations of genetamicin, a standardized culture of gentamicin sensitive *E. coli* was seeded on each of the plates using a sterile swap stick. The process was repeated using each of the other 4 Enterobacteriaceae isolates identified. All plates were incubated at 35°C for 18 h.

The above procedure was repeated using various concentrations of nalidixic acid in MHA as stated above. In this case, isolates that were sensitive to nalidixic acid were used. This test was performed in order to obtain an approximate concentration that would be used for selection of transconjugants. An approximate concentration of 60 and 90 ug/ml were obtained for gentamicin and nalidixic acid respectively.

Gentamicin resistance gene transfer by conjugation

The transfer of gentamicin resistance genes (Gm^r) among the isolates from UTI was investigated. Both donor and recipient isolates were subjected to plasmid curing using acridine orange and recipient isolates which lack replicative plasmids were used as recipient isolates in the conjugation experiment (Thompson, 1989; Kreuzer and Massay, 1996).

Using the method of Kreuzer and Massey (1996), as adopted from Thompsons (1989) design, recipient and donor strains were separately grown in 2 ml of nutrient broth, incubated overnight at 37°C. Meanwhile, a mating plate was prepared by supplementing MHA with nalidixic acid (90 µg/ml) and gentamicin (60 µg/ml). The plates were divided into 3 parts R, D and C.

Using a micropipette, 50 µl of donor's broth culture was transferred on to the agar at spots D and C, respectively. Using a different tip of the pipette another pair of 50 µl recipient broth culture was transferred on to spots R and C, respectively, on the same mating plate. The plate was then left to sit for about 5 min until all fluid has been absorbed unto the agar.

A sterile wire loop was used to streak out the inoculum and also subculture onto another MHA plate supplemented with gentamicin and nalidixic acid. All plates were incubated at 30°C for 24 h.

RESULTS

A total of 300 urine samples showed significant bacteriuria out of the 386 urine samples collected from patients attending the 3 hospitals in Mubi. The presence of lactose fermenting, gram-negative, non spore forming rods (coliform) were analysed from these 300 samples. 5 coliform bacteria species were identified; *E. coli, Klebsiella pneumonia, Klebsiella oxytoca, Enterobacter aerogenes* and *Citrobacter freundii.*

E. coli incidence in this study was shown in Table 1 with *E. coli* as the most predominant species isolated. A total of 191 samples were positive for UTI due to Enterobacteriaceae bacteria which account for 48.4% of total sample screened and 62.3% of samples with significant bacteriuria. Gender distribution showed that 30.2 and 69.3% were, respectively, positive for male and female.

All isolates identified were subjected to antibiotic susceptibility test. Isolates were found to be more resistance to ampicillin followed by ciprofloxacin, then amoxicillin-clavulanic acid, ofloxacin, perfloxacin, trimethoprim-sulphamethoxazole, gentamicin, cephalexin 31(16.2%), nalidixic acid and streptomycin (Table 2).

Results of antibiotic susceptibility test also indicated that *E. coli* expressed the highest rate of resistance to the entire antibiotics (Table 3).

A total of 33(17.7%) isolates were resistant to gentamicin with individual species resistance rates shown in Table 3.

A total of 29 gentamicin resistant donor isolates were subjected to the curing experiment of which 22(75.8%) were cured of gentamicin resistance. This implies that for the majority of the isolates subjected to curing, gentamicin resistance determinants were probably located on plasmids. 8 of the 22 isolates cured lost their entire resistance determinants.

The occurrence of plasmid-mediated resistance obtained among the selected donor isolates were shown in Table 4.

Intra-species and inter–generic transfer of Gm^r genes

Table 3. Percentage prevalence of gentamicin resistant isolates.

Bacteria isolate	Total no. of Isolates	No. of isolates resistant to gentamicin (Gm^r) (%)
E. coli	99	17 (17.2%)
K. pneumoniae	47	9 (19.1%)
K. oxytoca	6	1 (16.7%)
E. aerogenes	19	3 (15.7%)
C. freundii	21	3 (14.3%)

Table 4. Plasmid curing of donor isolates with acridine orange (0.1 mg/ml).

Bacteria isolate	Total No. subjected to curing	No. cured only *Gm*^{r}	No. cured of all makers	No. with plasmid
E. coli	14	07	03	10 (71.4%)
K. pneumoniae	08	05	02	07 (87.5%)
E. aerogenes	03	02	01	03 (100%)
C. freundii	04	-	02	02 (50%)

were carried out on the isolates. Results obtained from intra-species transfer showed that 4 (28.5%) out of the 14 *E. coli* used as donor transferred Gm^r gene to *E. coli* GH10 and *E. coli* GH38. Curing experiment results showed that donor *E. coli* isolates GH63, GH83 and BK167 probably had Gm^r gene(s) on the chromosome, yet transferred their genes to recipient isolates; *E. coli* GH10, GH38, GH76 GH41, NL135 and NL117 was observed. 6 (42.9%) of the 14 donor *E. coli* isolates transferred their Gm^r gene(s) to recipient *E. coli* NL117 and 10 (71.4%) of donor *E. coli* isolates transferred Gm^r gene(s) to *E. coli* GH41, NL101, while 7 (50%) donor *E. coli* transferred Gm^r to *E. coli* GH76, NL117 and NL135. No transfer of *Gm*^{r} genes was observed from donor isolates *E. coli* BK177 and *E. coli* BK 180; although curing experiments indicated that their Gm^r gene(s) are likely located on plasmids. Out of the 8 *K. pneumonia* used as donor isolates, only 4 (50%) *K. pneumonia* transferred Gm^r gene to *K. pneumonia* BK149 and 3 (37.5%) transferred Gm^r gene to *K. pneumonia* BK188. Transfer was not observed from *K. pneumonia* GH78 and *K. pneumonia* GH50 despite the fact that the curing experiments indicated that Gm^r is located on plasmid. Similarly 1 (33.3%) of *E. aerogenes* transferred its Gm^r gene to *E. aerogenes* GH6 and *E. aerogenes* GH75 while only 1 (25%) *C. freundii* (GH55) transferred its Gm^r gene to recipient *C. freundii* BK189 (Table 5).

Inter-generic transfer results are shown in Table 6. However, No transfer of Gm^r gene occurred in the following cases: from donor isolate *E. coli* BK177 and BK180 despite the fact that these isolates seem to have Gm^r gene on plasmids; from *K. pneumonia* GH78 and GH50 although the curing experiment showed that Gm^r gene is located on plasmids in these isolates; from *E. aerogenes* to *E. coli* NL117, *K. pneumonia* BK181 and *C. freundii* BK189; from *C. freundii* to *E. coli* GH76, GH38 and *E. aerogenes* GH75. Despite the fact that curing experiment indicated that Gm^r gene in *C. freundii* GH55 is probably located on the chromosome, *Gm*^{r} gene transfer was observed from this donor isolate to *K. pneumonia* BK181, *E. coli* GH41, *E. coli* GH10 and *C. freundii* BK189. However transfer of Gm^r gene was not observed from *C. freundii* BK143 that had its Gm^r likely located on plasmid.

DISCUSSION

UTI due to the Enterobacteriaceae bacteria and transfer of gentamicin resistance genes among the Enterobacteriaceae isolates were investigated among outpatients from 3 hospitals in Mubi. The most predominant isolates obtained were *E. coli* (51.5%) and *K. pneumonia* (24.4%). These findings agree with the reports of Mbata (2007) and Dytan and Chua (1999) which indicated that gram-negative bacteria mostly *E. coli* and *K. pneumonia* are the commonest pathogens isolated in patients with UTI.

There was a significant difference between mean UTI in male and female (at $p > 0.05$) in this study. The high prevalence of UTI in female may be due to gender related factors such as the anatomy of the female genital tract (Gupta et al., 2001; Michael and Loretta, 2004). This observation agrees with other reports which showed that UTI are more frequent in female than males (Ijah, 1996; Mbata, 2007). Nwanze et al. (2007) showed that there was no significant difference between the number of male and female patients with UTI in a study conducted in Okada village at $p < 0.05$. The rate of resistance of isolates to gentamicin in this study is comparable to that of other workers. Mbata (2007) reported gentamicin resistance rates of 10.6% in *E. coli*, 29% in *K. pneumoniae* and 10% in *C. freundii;* Ijah (1996) reported

Table 5. Intra – species transfer of Gm^r genes.

Donor isolate (Gm^r Nal^s)	Recipient (Gm^s Nal^r)	No. of transconjugant identified (%)
E. coli (14)	*E. coli* GH 10	4 (28.5)
E. coli (14)	*E. coli* GH38	4 (28.5)
E. coli (14)	*E. coli* GH41	6 (42.9)
E. coli (14)	*E. coli* GH76	7 (50.0)
E. coli (14)	*E. coli* NL101	6 (42.9)
E. coli (14)	*E. coli* NL117	7 (50.0)
E. coli (14)	*E. coli* NL135	7 (50.0)
K. pneumonia (8)	*K. pneumonia* BK149	4 (50.0)
K. pneumonia (8)	*K. pneumonia* BK181	3 (37.5)
E. aerogenes (3)	*E. aerogenes* GH6	1 (33.3)
E. aerogenes (3)	*E. aerogenes* GH 75	1 (33.3)
*C. fruendii (*4)	*C. freundii* BK189	1 (25.0)

Table 6. Inter – generic transfer of Gm^r gene.

Donor isolate (Gm^r Nal^s)	Recipient (Gm^s Nal^r)	No. of transconjugant identified (%)
E. coli (14)	*K. pneumonia* BK 149	7 (50.0)
E. coli (14)	*K. pneumonia* BK 181	6 (42.9)
E. coli (14)	*Klebsiella oxytoca* GH 68	6 (42.9)
E. coli (14)	*E. aerogenes* GH6	5 (35.7)
E. coli (14)	*E. aerogenes* GH 75	5 (35.7)
E. coli (14)	*C. freundii* BK 189	3 (21.4)
K. pneumonia (8)	*E. coli* GH10	4 (50.0)
K. pneumonia (8)	*E. coli* GH38	3 (37.5)
K. pneumonia (8)	*E. coli* GH41	4 (50.0)
K. pneumonia (8)	*E. coli* GH76	5 (62.5)
K. pneumonia (8)	*E. coli* NL101	4 (50.0)
K. pneumonia (8)	*E. coli NL117*	3 (37.5)
K. pneumonia (8)	*E. coli* NL135	3 (37.5)
K. pneumonia (8)	*K. oxytoca* GH68	2 (25.0)
K. pneumonia (8)	*E. aerogenes* GH6	2 (25.0)
K. pneumonia (8)	*E. aerogenes* GH75	3 (37.5)
K. pneumonia (8)	*C. freundii* BK189	2 (25.0)
E. aerogenes (3)	*E. coli* GH10	1 (33.3)
E. aerogenes (3)	*E. coli* GH38	1 (33.3)
E. aerogenes (3)	*E. coli* GH41	1 (33.3)
E. aerogenes (3)	*E. coli* GH76	1(33.3)
E. aerogenes (3)	*E. coli* NL101	1 (33.3)
E. aerogenes (3)	*E. coli* NL117	0 (0.0)
E. aerogenes (3)	*E. coli* NL135	1 (33.3)
E. aerogenes (3)	*K. pneumonia BK149*	1 (33.3)
E. aerogenes (3)	*K. pneumonia* BK181	0 (0.0)
E. aerogenes (3)	*K. oxytoca* GH68	1 (33.3)
E. aerogenes (3)	*C. freundii* BK189	0 (0.0)
C. freundii (4)	*E. coli* GH10	2 (50.0)
C. freundii (4)	*E. coli* GH38	0 (0.0)
C. freundii (4)	*E. coli* GH 41	1 (25.0)
C. freundii (4)	*E. coli* GH76	0 (0.0)

Table 6. Contd.

C. freundii (4)	*E. coli* NL101	2 (50.0)
C. freundii (4)	*E. coli* NL117	2 (50.0)
C. freundii (4)	*E. coli* NL135	1 (25.0)
C. freundii (4)	*K. pneumonia* BK149	1 (25.0)
C. freundii (4)	*K. pneumonia* BK181	1 (25.0)
C. freundii (4)	*K. oxytoca* GH68	2 (50.0)
C. freundii (4)	*E. aerogenes* GH6	1 (25.0)
C. freundii (4)	*E. aerogenes GH* 75	0 (0.0)

gentamicin resistance rate of 25% in *K. pneumoniae*;Gales et al. (2000) reported an overall gentamicin resis-tance rate of 25.1% of UTI isolates in Latin America while Nwanze et al. (2007) reported gentamicin resistance of 59% in *K. pneumoniae* and 46% in *E. coli,* while Bartoloni et al. (2006) reported an overall gentamicin resistance rate of 21% in *E. coli* isolated from children in 4 urban areas in Peru and Bolivia. The differences in results by different investigators could be attributed to differences in the number of positive cases of urinary tract infection examined and differences in geographical region. Such differences could affect the prevalence of one organism and antimicrobial resistant pattern. In addition, different social and human activities could account for the observed differences.

The most useful antibiotics in this study were streptomycin, nalidixic acid, cephalexin and gentamicin. This is because large percentage of isolates mostly *E. coli* and *Klebsiella species* were highly inhibited by these antibiotics. High resistance of the Enterobacteriaceae bacteria to antibiotics observed in ampicillin, ciprofloxacin, amoxicillin and clavulanic acid, is not a surprise because these drugs are usually recommended for the treatment of UTI and are readily available from diverse sources. This could have led to gross abuse of the drugs by individuals, resulting in diminished effectiveness of the drugs. Agricultural use of antibiotics to enhance productivity in livestock has also led to increased rate of resistant species dissemination among the Entero-bacteriaceae (Pelczer et al., 1986). High resistance of the isolates to ciprofloxacin as indicated by this study could also be due to overuse of chloroquine to prevent and treat malaria. Mark (2008) reported that *E. coli* strains resistant to ciprofloxacin were detected in the digestive tracts of villagers from rain forest community in Guyana, despite that they had never been given the drug. Most of the villagers however had been given chloroquine, a drug closely related to ciprofloxacin. In view of the wide usage of ciprofloxacin, resistance to this antibiotic and other fluoroquinolones could constitute an important public health problem in area where malaria is endemic. Therefore there is need to prevent malaria using integ-rated approach coupled with development of effective vaccine so that humans will not end up creating more problems in an attempt to solve one. Another possibility of resistance development is by horizontal transfer of the resistance determinants which have been shown to be common among members of Enterobacteriaceae (Osterblad, 2000; Lawrence, 2005; Tenover, 2006).

Gentamicin resistance among the Enterobacteriaceae isolates is still manageable as indicated by the results of this study. The curing experiment of the 29 donor isolates showed that gentamicin resistance in 22 (75.8%) of the isolates were plasmid mediated. This implies that resistance trait may be easily transferred among bacterial population through a conjugational process.

Series of researches have shown that dissemination of resistance genes mediated by plasmids among bacteria can occur by conjugation (Aluyi and Akortha, 2002; Diaz et al., 2006; Shoemaker et al., 2001; Ferguson et al., 2002). Sequencing and PCR experiments confirmed that Gm^r genes are present on mobile genetic elements that can facilitate their horizontal transfer among bacteria (Heuer, 2002; Diaz et al., 2006). Ferguson et al. (2002) reported that gene transmission between bacteria can occur inside animal cells by conjugation and the conjugation in cell may be as efficient as on agar plate. In this study, an *in vitro* intra-species transfer of Gm^r by conjugation was observed in *E. coli*, *K. pneumoniae*, *E. aerogenes* and *C. fruendii*; suggesting that the Gm^r determinant(s) in these isolates may be located on conjugative plasmids. However, transfer of gentamicin resistance determinant(s) was not observed in some isolates that had their Gm^r genes on plasmids. This is an indication that the Gm^r genes in these isolates are probably located on non conjugative plasmids. On the contrary, transfer was observed in some isolates that had their Gm^r genes on the chromosome. It is most probable that the Gm^r genes were located on transposable element or integron thus resulting in transfer function.

Inter generic transfer of Gm^r was also observed with high transfer rate from donor isolates to *E. coli*. This finding underscores the flexible ease with which *E. coli* accepts exogenous genes as also evident in its ability to acquire various virulent factors from other bacteria (e.g. *Shigella* sp.). The average intra species and inter generic transfer rates from this study were 41.1% and 34.8% respectively. Aluyi and Akortha, (2002) reported transfer efficiency of 33% from some enteric bacteria of diarrhea origin to *E. coli* (UB5201). The inter generic transfer rate

in the present study is comparatively lower than the intra specific transfer. Lower rates of inter generic Gm^r transfer could be as a result of fertility inhibition, incompatibility, inability to synthesize adhesion and/or narrow host range (Tolmasky, 1990).

The presence of plasmid mediated gentamicin resistance among coliform isolates and evidence of the transferability of Gm^r gene between its genera and species implies that under favorable conditions, conjugal transfer of R plasmids could occur *in vivo*. Besides, possibility of further transfer to *Salmonella* sp., *Proteus* sp. and other pathogenic bacteria should not be ignored. This is because several studies have supported the flexible ease of genetic transfer existing among the enterobacteriaceae family (Wang et al., 2004; Yukata et al., 2004).

Current knowledge about antibiotic resistance in urinary tract isolates in Mubi is limited, although resistance prolixferation in pathogens is a global problem. To main-tain effective therapy against urinary tract isolates, it is imperative to monitor susceptibility data as resistant infectious pathogens is continually emerging. It is pertinent to note that the race to develop antibiotics to overcome the resistance mechanism is one that humans may never win, but the resistance trends should be kept under check through intensive research and antibiotic resistance surveillance. This could lead to novel and alternative drug therapies and also implementation of preventive measures on the proliferation of antibiotic resistance among pathogenic bacteria.

In line with similar initiative in Europe, Asia and America, the government is encouraged to set up surveillance centers responsible for evaluating the emergence of antimicrobial resistance in pathogens in the 6 geopolitical zones since none exist in the country presently (Yah et al., 2006). This will go a long way in monitoring emerging infectious pathogens and multi drug resistance profiles among clinical isolates.

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