Scientific Research and Essay Vol. 3 (1), pp. 028-034, January 2008
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2008 Academic Journals

Full Length Research Paper

Plasmid-borne antibiotics resistant markers of *Serratia marcescens*: an increased prevalence in HIV/AIDS patients

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Accepted 20 November 2007

This study was carried out to evaluate the resistant pattern of multi-drug resistant strains of *Serratia marcescens* associated with HIV/AIDS infected individuals in Nigeria. A total 1709 samples (1323 urine and 406 diarrhea stool) were collected from individuals living with HIV/AIDS over a three year period and screened for plasmid-mediated *S. marcescens* susceptibility profile to antibiotics. These individuals had been confirmed HIV positive and their CD4 cells enumerated using the Partec cyflow (Partec GmbH, Germany). The CD4 values ranged between 14 and 812 cells/µl of blood. During this period, ninety-four (94) *S. marcescens* strains were isolated of which 38(40.4%) were from urine samples while 56(59.6%) were from diarrhea stool samples. The resistance patterns of the isolates varied ($p<0.05$) within all the antibiotics tested. The resistant genes were highly encoded on transferable plasmids with a varied frequency of 2×10^{-2} to 6×10^{-3} per donor cell by conjugation. Eight out of nine of the samples (88.9%) had multiple plasmids with molecular weights range between 1.14 and 6.0 kb. Curing experiment results showed that resistance genes were also chromosomally mediated. This indicates that there is a high antibiotic resistant genes marker among *S. marcescens* strains in HIV/AIDS individuals in Nigeria.

Key words: Plasmids Borne- *Serratia marcescens*-HIV/AIDS

INTRODUCTION

Serratia marcescens is one of the major nosocomial pathogen found associated with urinary and respiratory tract infections, endocarditis, osteomyelitis, septicemia, wound infections, eye infections (conjunctivitis) and meningitis (Dodson, 1968; CDCP, 1994; McNaughton et al., 1995; Dray-Spira et al., 2000; Ostrowsky et al., 2002; Bagattini et al., 2004; Ayush and Elizabeth, 2005; Jose et al., 2006). Transmission is mainly by direct contact, droplets, catheters, saline irrigation solutions and in other supposedly sterile solutions. Currently, *S. marcescens* have been found to be resistant to gentamicin, ofloxacin, ampicillin, chloramphenicol and amikacin (Yohei et al., 2004). Before 1951, the bacterium was thought to be non-pathogenic and because of the pigment it produces, it was used widely to trace bacterial transmission. In 1951 and 1952 the US Army carried out the "Operation Sea-Spray" to study wind currents that might carry biological weapons. Shortly thereafter, doctors noted a drastic increase in pneumonia and urinary tract infections with *S. marcescens* incriminated (Woodward and Clark, 1913; Wheat et al., 1951). However, the prevalence of *S. marcescens* in human diseases had been underestimated for years before the first known outbreak of nosocomial *S. marcescens* infections in 1951 (Woodward and Clark, 1913; Wheat et al., 1951). Since 1960, infections with this organism have been reported with increasing frequency (Ostrowsky et al., 2002; Lin-Hui et al., 2003).

Infections with opportunistic pathogens have been one of the hallmarks of the acquired immunodeficiency syndrome since the beginning of the epidemic. Little atten-

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tion has been given to the role of *S. marcescens* infection in complications associated with HIV infections. Urinary and gastrointestinal tracts infections account for a sizeable number of these opportunistic infections in hospitalized HIV-infected individuals in developing countries (Bernstein et al., 1985; Meyer et al., 1994; Okuda et al., 1984).

Also, bacterial resistance to several classes of antibiotics in HIV-infected individuals in Sub-Saharan countries is on the increase, ranging between 0.25 and 21% in some instances (Bernstein et al., 1985; Roilides et al., 1991; Spach and Jackson, 1999; Yah et al., 2004). The increased predisposition of HIV/AIDS patients to invasive bacterial isolates has been described but there are no detailed studies or literatures on the antibiotics susceptibility pattern (ASP) of *S. marcescens* in HIV/AIDS individuals. This study is therefore designed to look at the ASP of *S. marcescens* in HIV infected individuals in Nigeria.

METHODOLOGY

Sample collection and isolation of organism

One thousand three hundred and twenty three (1323) urine and 406 diarrheal stool samples were collected from confirmed long-term HIV/AIDS patients attending University of Benin Teaching Hospital (UBTH) Nigeria from May 2002 to May 2004 and examined for *S. marcescens* susceptibility profile to plasmid-mediated antibiotics. Also 703 urine and 231 stool samples were collected from non infected HIV/AIDS patients as control attending the same hospital (UBTH). The patients were screened for their human Immunodeficiency virus (HIV) antibodies using the Wellcozyme ELISA technique as recommended by Wellcome while the cytometer was used for the CD4 cells count (Partec Cyflow Germany). The urine and diarrheal stool samples were inoculated aerobically on nutrient agar, MacConkey agar and pronounce red pigment formation on nutrient agar slants at 37°C for 24 to 48 h. The colonies of each representative isolates were then characterized using standard bacteriological methods earlier described (Cowan and Steel, 1993; Cheesbrough, 2000).

Antibiotic susceptibility

The antibiotic resistance patterns of the isolates were determined by inoculating on Oxoid-Mueller-Hinton agar (Difco Laboratories, Detroit, Mich USA) plates, using the disc diffusion method. The inocula were prepared directly from an over night agar plate adjusted to 0.5 McFarland standard. Commercially prepared antibiotics impregnated discs (Optun Laboratories Nigeria Limited., Nigeria), containing the following antibiotics: ampicillin (Am) 30 µg, gentamicin (Gn) 10 µg, nalidixic acid (Na) 30 µg, norfloxacin (Nb) 30 µg, nitrofurantoin (N) 100 µg, pefloxacin (Pef) 5 µg, cotrimoxazole (Co) 50 µg, ciprofloxacin (Cip) 5 µg, and chloramphenicol (C) 10 µg were aseptically placed on the inoculated plates and incubated overnight. The zones of inhibition were measured and interpreted according to NCCLS (NCCLS, 2000) after incubation at 37°C for 24 h. *Serratia* strains that showed resistance to three or more antibiotics were taken to be multi- antibiotic resistant and were preserved for further analyses.

Genetic transfer experiments

The conjugation method was carried according to Wang et al. (2004) and Yukata et al. (2004) with *Escherichia coli* K rifampicin resistant (EJRifr) as the recipient. Resistance transfer markers were selected by a combination of antibiotics to which the transconjugants were resistant but to which the parent strains were sensitive. Antibiotic sensitivity test were further carried out to determine the presence of resistance markers of the donor and the recipient. The frequencies of transfer were determined by dividing the number of transconjugants by the number of donor cells according to Wang et al. (2004).

Curing experiments

Curing of the strains was carried out using the modification of Olukoya and Oni method (1990). The *Serratia* cells were cured by treating the cells with 10% sodium dodecyl sulfate (SDS). The colonies were then sub-cultured onto Mueller Hinton agar (Difco Laboratories, Detroit, Mich USA) plates and test run for their respective antibiotic sensitivity patterns as previously described. Some of the bacteria were sensitive while some were resistant. Absence of growth in Mueller Hinton agar was indicative of plasmids-mediated resistance while growth in Mueller Hinton agar was indicative of chromosome-mediated.

Plasmids isolation experiments

Plasmids isolation was carried out based on the rapid alkaline extraction procedures for screening of recombinant plasmid DNA, using the method of Zhou et al. (1990). Agarose gel electrophoresis was used to resolve the extracted plasmids; standard DNA molecular weight marker II (0.12 - 23.1 kbp) of bacteriophage lambda Hind II (Roche Diagnostic GmbH) was used as standard DNA marker. Plasmid compositions of the semi purified clear lysates were determined by horizontal agarose gel electro-phoresis of Meyers et al. (1979). This included the use of 0.8% agarose slab gels in Tris borate EDTA (TBE) buffer. The gels were stained with 14 µl ethidium bromide for 45 min and then photo-graphed under *UV* light trans-illumination before comparing with the standard marker.

Data analysis

The Chi-square test and the student two-tailed t test were used. A difference was considered significant when P-value by the two-tailed was less than 0.05 ($P<0.05$). Results were expressed as mean standard deviation ($\overline{X} \pm S.D$).

The calculated values where then compared with the critical values at the appropriate degree of freedoms at a significant level of $P = 0.05$ (Ogbeibu, 2005).

RESULTS

A total of 1323 urine and 406 diarrhea stool samples were collected from HIV/AIDS patients for plasmid-mediated S*. marcescens* susceptibility profile to antibiotics (Table 1). A total of 94 *S. marcescens* strains were isolated; 38(40.4%) were from urine samples while 56(59.6%) were from diarrhea tool samples of HIV/AIDS infected patients (Table 1). On the other hand, 5 *S. marcescens* were recovered from non infected HIV/AIDS case-group

Table 1. Percentage (%) occurrence of resistant *Serratia marcescens* from a University Teaching Hospital

	Types of antibiotics									
Source	**Total No. of isolates**	**Am**	**Co**	**N**	**Na**	**Cip**	**Nb**	**Pef**	**Gn**	**C**
Urine (n = 204)	38(40.4%)	26(68%)	20(53%)	19(50%)	8(21%)	6(16%)	8(21%)	6(16%)	18(47%)	19(50%)
Diarrheal Stool (n = 233)	56(59.6%)	48(86%)	32(57%)	19(34%)	17(30%)	5(9%)	16(29%)	8(14%)	16(29%)	21(38%)

Key: () – Percentage; Am = Ampicillin, Co – Cotrimoxazole, N = Nitrofurantoin, Na – Nalidixic acid, Cip – Ciprofloxacin, Nb – Norfloxacin, Pef – Pefloxacin, Gn - Gentamicin, C - Chloramphenicol.

Table 2. CD4count (cells/□l of blood) among HIV/AIDS patients from University of Benin Teaching Hospital (UBTH) against *Serratia marcescens* infections.

Age group (years)	CD4count (cells/µl of blood) (× ± *D*)
1-10 (n = 720)	14 – 348 (106.4 ± 79.4)
11-20 (n = 213)	16 – 812 (190.9 ± 290.0)
21-30 (n = 428)	102 – 628 (208.0 ± 145.4)
31-40 (n = 261)	34 – 347 (126.0 ± 134.3)
≥41 (n = 87)	16 – 528 (102.1 ± 185.0)

counterparts; 2 from urine and 3 from stool samples respectively. The CD4 cells count of the HIV/AIDS patients ranged between ≤14 and ≥812/ µl of blood and varied with different age groups (Table 2). The results revealed a CD4 cells count of 14 to 348/ µl (106.4 ± 79.4); 16/ µl to 812 (190.9 ± 289.8); 102 to 628/ µl (208.0 ± 145.4); 34/ µl to 347/ µl (126.0 ± 134.3) and 16 cells/ µl to 528 cells/ µl (102.1 ± 185.0) respectively for age groups 1 - 10, 11 - 20, 21- 30, 31 - 40 years, and 41 years and above, before commencement of antiretroviral chemotherapy (Table 2). There was no significant differences ($P>0.05$) between the percentage prevalence of HIV/AIDS infected individuals within the age groups. The CD4 count of all the non infected HIV/AIDS individuals ranged from 758 to >1000/µl. Thirty out of the thirty-eight strains of HIV/AIDS from urine (78.9%) and 39 out of the 56 isolated from diarrheal stool (69.6%), were resistant to 3 or more classes of antibiotics. The resistance pattern of the isolates varied ($P<0.05$) in all the antibiotics tested. There was no significant differences between ($P>0.05$) the resistant pattern of strains from urine and diarrheal stool samples of HIV/AIDS individuals. The strains were highly resistant to ampicillin followed by cotrimoxazole but were least resistant to norfloxacin, pefloxacin and ciprofloxacin. On the other hand, all the 5 isolates from the non infected HIV/AIDS case controlled group were sensitive to all the antibiotics used.

The resistant genes were highly encoded on transferrable plasmids with a varied frequency of 2×10^{-2} to 6×10^{-3} per donor cells by conjugation as shown in (Table 3). All the strains were able to transfer ampicillin markers to their recipients followed by nitrofurantoin, gentamicin, cotrimoxazole and nalidixic acid. Ampicillin and chloramphenicol were also highly mediated by chromosomal genes followed by the quinolones (norfloxacin, ciprofloxacin and pefloxacin). There was no significant differences ($P>0.05$) between chromosome carriage genes of strains from diarrhea and urine samples. The results also showed that one transconjugant strain out of nine (11.1%) had one plasmid while eight out of nine (88.9%) had multiple plasmids with molecular weights ranging between 1.2 and 6.0 kb (Table 4). All the donor cells were able to transfer ampicillin to their transconjugants; followed by cotrimoxazole and gentamicin. The plasmids DNA bands of the donors and transcon-jugants are as shown in Plates 1 and 2.

DISCUSSION

Several studies have shown that there is a synergy between intestinal opportunistic pathogens and HIV (Naoki and Susumu, 1995; Spach and Jackson, 1999). It has also been shown that these pathogens breakdown the mucosal barrier, thereby permitting easier accesses of the HIV to blood and consequently to the T helpers' and immune cells (Roitt 1989). The present study has shown such detrimental association between HIV/AIDS and *S. marcescens* of the urinary and gastrointestinal tract. The HIV weakened and reduced the host immune system facilitating opportunistic pathogens to thrive. Forty two percent (42.1%) of the individuals in this study were bet-

Table 3. Detection of *Serratia marcescens* resistant transconjugants from a University Teaching Hospital (UBTH).

Code	Resistant Markers of donors (DN)	EJRif^r + DN	Inference	Frequency	Resistant markers of transconjugants
SU112	GnAmNC	Growth	Significant	0.20×10^{-1}	Am-N-Co
SU022	CipNaCAmNPefNbCoGn	Growth	Significant	0.21×10^{-1}	Am-Gn-N-Co-Na
SU026	CAmNCip	Growth	Significant	0.21×10^{-2}	Am-N-C
SU0401	NaAmNCipCoNbPef	Growth	Significant	0.20×10^{-1}	Co-Am-N-Gn
SU226	CAmNGn	Growth	Significant	0.22×10^{-2}	C-Am-N-Gn
SU032	CipNNaCAmPefCoGn	Growth	Significant	0.20×10^{-1}	N-Am-Co-Gn
SU033	NAmC	Growth	Significant	0.21×10^{-1}	Am-N
SU412	CipNPefNbCoGnNaCAm	Growth	Significant	0.60×10^{-2}	Gn-Na-N-Am-Co
SU317	NPefNbCoGnNaCAm	Growth	Significant	0.60×10^{-1}	Co-Am-Gn

Table 4. Antibiotics resistant pattern of transconjugants and chromosome (after curing) from a University Teaching Hospital a 3 year study.

Code of isolates	Source	Resistant marker spectrum of donor/ before curing	Plasmid profile of donor (kb)	Plasmid profile of transconjugant (kb)	Resistant marker spectrum of transconjugant	Resistant Spectrum after Curing
STU112	Diarrhea	GnAmNC	3.0, 4.3,6.0	4.3, 6.0	Am-N-Co	Am-N
STU022	Urine	CipNaCAmNPefNbCoGn	1.6,3.0, 4.7	-	Am-Gn-N-Co-Na	Nb-Gn-Cip
STU026	Urine	CAmNCip	3.0, 3.4, 4.5	3.4, 4.5	Am-N-Co	Cip-C-Am
STU0401	Urine	NaAmNCipCoNbPef	3.0, 5.5, 5.6	5.5, 5.6	Co-Am-N-Gn	N-Am-Nb-Co-Pef
STU226	Diarrhea	CAmNGn	3.0 4.7, 5.5	4.7, 5.5	Am-N-Gn	Am-C
STU032	Urine	CipNNaCAmPefCoGn	2.5,3.0,4.3,4.7	3.0, 4.3, 4.7	N-Am-Co-Gn	Pef-Gn-Am-Co
STU033	Urine	NAmC	3.0 3.4, 4.5	2.5, 3.4	N-Am	Am-C
STU412	Diarrhea	CipNPefNbCoGnNaCAm	2.5, 3.0,4.4, 4.7	3.0,4.4, 4.7	Gn-Na-N-Am-Co	Cip-Pef-C
STU317	Diarrhea	NPefNbCoGnNaCAm	1.14, 2.5,3,4.6,4.7	3, 4.6,4.7	Am-Co-Gn	Cip-C-Nb

ween the ages of 1-10 years. The vulnerability of HIV-infected children to invasive bacterial infections is far greater than that seen in immunocompetent, HIV-uninfected children and HIV-infected adults (Kwara et al., 2005). HIV-infected children in many developing nations suffer from malnutrition, inadequate medical care, and an increased incidence of co-infection with diseases such as tuberculosis, cytomegalovirus and syphilis (Pillay et al., 2001). Dray-Spira et al. (2000) had shown that in Malawi and Uganda, the median survival of HIV-infected children at 3 years of age in two pediatric cohorts was only 34%. The incidence of *S. marcescens* was high in this age group compared to age group 21 - 30 years, which accounted for 52.4% of those hospitalized. A similar finding by Manfredi et al. (2000) found that hospital-acquired *Serratia* species infection were more frequent than community-acquired infection and was significantly related to AIDS, neutropaenia, and sepsis. The persistent risk of developing *S. marcescens* and other opportunistic pathogens in gastrointestinal tract (GIT) and urinary tract infections (UTI) co-infected with HIV may be due to impaired immune system as compared to HIV uninfected individuals. The study illustrated *S. marcescens* to be co-linked as an important opportunistic pathogen in HIV infected patients. This is in accordance with other pathogens such as *Mycobacterium tuberculosis*, cytomegalovirus, malaria parasite (Kublin et al., 2005; Kwara et al., 2005) which have been found be co-associated and as an index for suspected HIV/AIDS patients during diagnosis in Africa.

Our results also showed an increased spectrum of antibiotics resistant pattern of *S. marcescens* in HIV/AIDS infected individuals. The antibiotic resistance pattern varied ($P<0.05$) within the age groups. Forty eight (86%) *S.*

marcescens isolates were resistant to ampicillin from diarrheal stool and 26(68%) resistant to ampicillin from urine samples. These results were similar to those of Manfredi et al. (2000) where they found that antimicrobial sensitivity testing of *S. marcescens* showed complete resistance to ampicillin but elevated susceptibility to second-and third-generation cephalosporins, aminoglycosides, quinolones, and cotrimoxazole. This was also supported by the fact that HIV complicates the treatment of infections by increasing mortality and morbidity during antibiotic treatment and also increasing the risk of recurrent infections after the treatment has been successfully completed. Antiretroviral therapy (ART) can potentially reduce mortality rate of recurrent infections of *S. marcescens*. This is because nevirapine, which is used as a first line regimen can be used, unchanged in the presence of antibacterial therapy, thereby improving the management of HIV/AIDS related infections. In developing countries self-prescription and mis-use/abuse of antibiotics is on the higher side because drugs in these countries are often sold in open markets and on buses by lay-persons (Okeke et al., 1999; Yah et al., 2006a). Moreover, since most of the patients often travel long distances and incur large expenses for medical care, they are unlikely to return for follow-up visits. In addition, the patients may be unable to read antibiotic labels. Some of the drugs are usually expensive; patients purchase incomplete regimens whenever possible and may discontinue treatment when the symptoms and signs disappear before the pathogen is eliminated (Lansang et al., 1990). Misuse of these antibiotics by HIV/AIDS patients and health personnel in clinical practice can provide selective pressure favoring resistant *S. marce-scens* strains; the inappropriate use increases the risk for selection and dissemination of antibiotic-resistant bacteria, which are often placed at a competitive advantage (Okeke et al., 1999; Manfredi et al., 2000; Levy, 2001).

The resistant pattern was also found to be highly transferable on R- plasmids and on chromosomal DNA as shown in Figures 2 and 3 . Other findings by Jun et al. (2006) show that four strains of *S. marcescens* isolated from urine specimens collected from inpatients on a cerebral surgical ward, showed identical Spel PFGE pattern, indicating that a single *S. marcescens* clone could caused nosocomial UTI over a 14 month period in the ward. These four strains were all resistant to ceftazidine. Their findings proposed that ceftazidine resistant *S. marcescens* strains were likely chromosomal variant with gene mutation(s) but were unclear about the clinical significant of extended- spectrum cephalosporins resistant *S. marcescens* harboring mutated chromosomal gene. Such nosocomial profile might be responsible for causing sub-lethal endemic infections throughout the year**.** Although there were no epidemic antibiotic resistance markers of any *S. marcescens* strains, reported during the course of the study, these results were similar to the plasmid-mediated resistance profile of *Proteus* species and *Pseudomonas* isolates in Nigerian Hospitals reported by Yah et al. (200b; 2007) where they obtained a wide range of transmissible plasmids among the pathogens with no reported cases of epidemic.

Our results have shown that ampicillin and chloramphenicol resistant *S. marcescens* genes were mainly chromosomally mediated. The *S. marcescens* chromosomal transfer genes could have resulted from the host chromosome mobilization. This is in agreement with the earlier reports of Sheikh et al. (2003) where they found that plasmid-mediated antibiotics resistance of indigenous *Klebsiella* could be eliminated from the bacterial cell with the use of chemicals, indicating chromosome mobilization as one of the sole antibiotic resistance gene transfer. The results therefore showed that the resistance to antibiotics was either chromosomally mediated, plasmid mediated or environmentally influenced. Non-transference of plasmids could have resulted from plasmids that were too heavy to be transferred (Kublin et al., 2005) or lost during successive sub-cultures of the isolates. Although plasmids can easily be lost through successive cultures and from old stored cultures (Yah et al., 2006b), the inability of some of the *S. marcescens* isolates to exhibit particular resistant pattern after curing indicated that resistance to some antibiotics were plasmids or chromosome mediated or both plasmid- and chromo-some-mediated.

Also, the sizes of resistant *S. marcescens* plasmid genes ranged from 1.14 to 5.6 kb. These plasmids were highly transferable to recipient cells with a high frequency 2×10^{-2} to 6×10^{-3} per donor cell. The high rate of transfer may be due to the high percentage of plasmids per bacterial cells within the population as earlier reported by Yutaka et al. (2004). However, Collatze et al. (1989) reported that R-plasmids sometimes can be transferred but cannot replicate within the recipient cell and the transconjugants cannot express the donor's transfer genetic factors. Yutaka et al. 2004) also reported that the R-plasmids factor during mating can be interrupted, with only the resistance transfer factor (RTF) portion transferred.

The results therefore suggest that conjugal transfer of R- plasmids might occur in the intestinal and urinary tracts which are the main sites of these strains. The possibility of further transfer of the resistant marker profiles to other pathogenic bacterial isolates such as *Salmonella, Klebsiella, Proteus* and diarrhoeagenic *E.coli* should not be ignored. These high frequencies of transfer support the flexible ease of genetic transfer existing among the enterobacteriaceae family (Wang et al., 2004; Yukata et al., 2004; Olukoya and Oni, 1990; Yah et al., 2007b).

Transferable multi-antibiotic resistant bacterial genes have been reported on transmissible plasmids (Yah et al., 2006b; 2007). The current studies confirmed that *S. marcescens* cells carry a vast diversity of transferable plasmids mediated-antibiotics resistance (Tables 3 and 4).

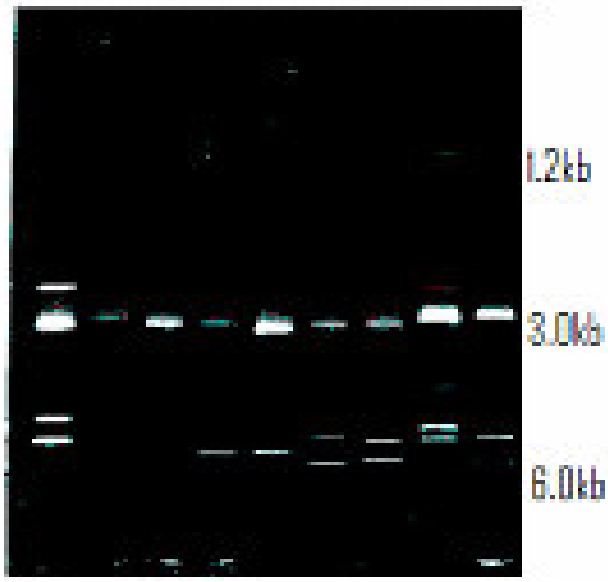

Figure 1. Agarose gel electrophoresis of plasmids DNA isolated from donor Serratia strains subjected in 0.8 g agarose, stained with 14 µl ethidium bromide (Zhous et al., 1990).

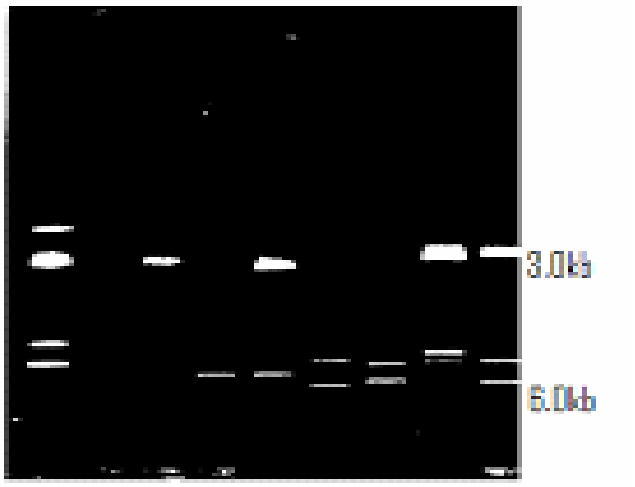

Figure 2. Agarose gel electrophoresis of plasmids DNA subjected in 0.8 g agarose, stained with 14µl ethidium isolated from transconjugants Serratia strains. Bromide (Zhou et al., 1990).

Some of the *S. marcescens* isolates had one plasmid, while some had multiple plasmids (Figures 1 and 2). This is in accordance with earlier reports by Wang et al. (2004) while detecting transferable emerging plasmid-mediated quinolone resistance associated with the quinolone gene in *Klebsiella pneumoniae* in the United States, where they confirmed a vast diversity of transferable plasmid-mediated antibiotics resistance genes among isolates. Similar results were also reported by Yah et al. (2007b) where they found that multi-resistant *Proteus* species had transferable plasmids-mediated resistance. The results also indicated that antibiotic resistance plasmids carried by Nigerian clinical isolates lack discernible evolutionary lineages; instead they demonstrate the distribution of similar resistance profiles in a diverse transferable genetic backgrounds.

The study therefore suggests further investigations of *S. marcescens* as a co-associated index for HIV co-infected patients as in the case of tuberculosis, *Pneumocystis carinii* pneumonia and cytomegalovirus than in non-HIV/AIDS un-infected individuals. *S. marcescens* infections according to Manfredi et al. (2000) may be responsible for appreciable morbidity among patients with HIV disease, especially when a low CD4 cell count, neutropaenia, and hospitalization are present. The clinician and the microbiologist facing a severely immuno-compromised HIV-infected patient with a suspected GIT and UTI bacterial infections could consider the *Serratia* species as co-complications as well. In fact, a rapid diagnosis and an adequate and timely treatment can avoid the disease relapses and reduce the morbidity and mortality.

ACKNOWLEDGEMENT

We wish to thank the staff of the Nigerian Institute for Medical Research (NIMR) Yaba Lagos, Nigeria especially Dr (Mrs.) S I. Smith of the Biotechnology Unit for their assistance.

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