African Journal of Biotechnology Vol. 6 (15), pp. 1757-1762, 6 August 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Widespread plasmid resistance genes among *Proteus* species in diabetic wounds of patients in the Ahmadu Bello university teaching hospital (ABUTH) Zaria

Yah, S. C[1], Eghafona, N. O[1], Oranusi, S.[2] and Abouo A. M.[1]

[1]Department of Microbiology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Accepted 18 May, 2007

Plasmids have been known to play a major role in the dissemination of antibiotics resistance genes in a microbial population. In this background, 148 *Proteus* species comprising of 97 *Proteus mirabilis* and 51 *Proteus vulgaris* were isolated from diabetic wounds. Seventy-six strains had varied multi-drug resistance (MDR) pattern encoded on transferable plasmid gene with a very high frequency (2 x10^{-4} to 4 x 10^{-2} per donor cell) by conjugation. 34% of the strains lost the antibiotic resistance plasmids marker after sodium dodecyl sulfate (SDS) mediated curing. The rest of the plasmid markers were non transferable. The results indicated that plasmids carry varied dissemination of antibiotics resistance markers to distant recipient cells, indicating clonal transfer among bacterial strains.

Key words: Resistant, plasmids, *Proteus,* diabetic, wounds.

INTRODUCTION

Diabetic wound lesions are a major medical, social and economic problem and are the leading cause of hospitalization for patients with diabetes (Ravisekhar et al., 2006). Diabetes is a disorder in which the pancreas does not produce enough insulin for the body need (Frier et al., 1999; Nelson, 2006). It is one of the world's major important health complications as well as a significant factor in the cost of inpatient treatment, lost of lives, disability and a reduction in life expectancy (Kumar and Clark, 2002). In Nigeria, there are over 10 million diabetic patients, half of which are unaware that they do have the disease with the highest prevalence > 30 years of age. Diabetic wounds/or foot ulcers and infections can lead to amputation of the foot or leg and one out 15 diabetic patients requires a limb amputation during their lifetime (Frier et al., 1999; Unachukwu et al., 2005). According to Motta et al. (2003) and Raviskhar et al. (2006) several enterobacteria and Gram-positive bacteria have been found to be associated with diabetic foot ulcers; therefore this should be a matter of great concern for those who treat and rehabilitate diabetic wounds.

Proteus species are part of the gram-negative bacilli found implicated in diabetic wounds along with *Escherichia, Klebsiella, Enterobacter,* and *Serratia* species (Goldstein et al., 1996; Motta et al., 2003; Jun et al., 2006; Raviskhar et al., 2006). According to the reports of Raviskhar et al. (2006) *Proteus* species are found to be most dominant Gram-negative isolates in diabetic wounds, followed by *Escherichia coli* and *Pseudomonas* species whereas *Staphylococcus aureus* and *Enterococcus* species are the most predominant Gram-positive isolates from diabetic wounds. *Proteus* species are found in multiple environmental habitats, including long-term care facilities and in hospitals. They are easily isolated from individuals in long-term care facilities, urinary tract, hospitals as well as from patients with underlying diseases or compromised immune systems (Louie et al., 1976; Cheesbrough, 2000). *Proteus* causes clinically significant infections and is often difficult to eradicate from the host especially in individuals with complicated foot ulcers, catheterization or with functional abnormallities of the urinary tract (Motta et al., 2003; Asad and Amna, 2004; Adel et al., 2005; Raviskhar et al., 2006).

Proteus colonizing the intestinal tract and wounds vary

*Corresponding author. E-mail: yahclar@yahoo.com. Phone: +2348053336108 or +2348063418265.

in their carriage of genes encoding antibiotic resistance (Asad and Amna 2004; Yah et al., 2004). However, the routine use of antimicrobial agents in both human and veterinary medicine has resulted in widespread antibiotic resistance and the development of antibiotic resistance genes especially within and between the Gram-negative bacteria (Ferber, 1998; Enabulele et al., 2006). With the presence of antibiotics selective pressure, these resistant *Proteus* species tend to persist, enabling the organism to cause extra infections such as septicemia (Prescott et al., 2001). The overall goal of the study is to determine the antibiotic susceptibility profiles of *Proteus* species from diabetic wounds and to assess the potential ability of the transferable antibiotics resistant plasmids markers of *Proteus* species from diabetic wounds of diabetes in the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria. This will enhance the epidemiological studies, empirical treatment and reduce the risks of diabetic wound complications.

METHODS

Isolation

A total of 148 *Proteus* species comprising of 97 *Proteus mirabilis* and 51 *Proteus vulgaris* were isolated from 168 diabetic wounds of diabetes attending ABUTH from 2003 to 2005 to examine their susceptibility plasmids profile to antibiotics. Specimens were obtained from both out and hospitalized patients. The specimens were collected with sterile swabs and inoculated aerobically on nutrient agar, blood agar and MacConkey agar (Cheesbrough, 2000) at 37^{o}C for 24 to 48 h. The bacterial species were identified by conventional biochemical tests as described by Cowan and Steel (1993). They were further sub-cultured and stored on nutrient agar slants at 4^{o}C for further analysis.

Antibiotic susceptibility

The antibiotic resistance patterns of the isolates were determined, using the disc diffusion method inoculated on Oxoid-Mueller-Hinton agar (Difco Laboratories, Detroit, Mich). The inocula were prepared directly from an over night agar plates adjusted to 0.5 McFarland standard of National Committee for Clinical Laboratory Standards, (NCCLS- 2000). The antimicrobial agents tested were ampicillin (Am) 30µg, gentamicin (Gn) 10µg, nalidixic acid (Na) 30µg, norfloxacin (Nb) 30µg, nitrofurantoin (N) 100µg, pefloxacin (Pef) 5µg, cotrimoxazole (Co) 50µg, cefotaxime (Ce) 5µg, ciprofloxacin (Cip) 5µg, and chloramphenicol (C) 10µg. These were aseptically placed on the inoculated plates and incubated overnight. The zones of inhibition were measured and interpreted according to NCCLS (2000).

Genetic transfer experiments

The conjugation method was carried according to Wang et al. (2004) and Yukata et al. (2004) with *E. coli* K-12J53-2 (F^{r} promet) Rif^{r} as the recipient. Resistance transfers were selective for by using a combination of antibiotics to which the transconjugants were resistant but to which the parent strains were sensitive. Antibiotic sensitivity tests were then carried out to determine the presence of the resistance markers of the donor and the recipient. Frequency of transfer was determined by dividing the number of transconjugants by the number of donor cells.

Curing experiments

To isolate the cured bacteria, modifications of Olukoya and Oni (1990) method was used. This was carried out by treating the cells with sodium dodecyl sulfate (SDS). The colonies were then sub-cultured onto Mueller Hinton agar (Difco Laboratories, Detroit, Mich) plates and test run for their respective antibiotic sensitivity patterns as previously described. Some of the bacteria were sensitive while some were still resistant. Absence of growth on 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 rapid alkaline extraction procedures for screening of recombinant plasmid DNA, according to Birnboim and Doly (1979) and Zhou et al. (1990) methods. Agarose gel electrophoresis was carried out to resolve the extracted plasmids with standard DNA molecular weight marker II (0.12-23.1 kbp; bacteriophage lambda HindIII Roche Diagnostic GmbH).

RESULTS AND DISCUSSION

The study revealed that 156 (92.9%) of the study subjects were males whereas the remaining were females (7.1%) with a mean age of 43.86 ± 9.22 years. A total of 148 *Proteus* species comprising of 97 *P. mirabilis* and 51 *Proteus vulgaris* were isolated from diabetic wounds of diabetes patients attending Ahmadu Bello University Teaching Hospital Zaria, Kaduna State, Nigeria as shown in Table 1. Apart from *Proteus* species, other bacterial isolates obtained were *S. aureus*, *E. coli, Pseudomonas aeruginosa, Klebsiella* species and *Staphylococcus epidermidis*. The susceptibility pattern of the *Proteus* species to different antibiotics varied ($p<0.05$) as shown in Table 1a where the isolates were highly resistant to ampicillin 60 (40.5%) and least resistant to cefotaxime 15 (10.1%), ciprofloxacin 9 (6.1%) and pefloxacin 7 (4.7%). There was no significant different ($P>0.05$) between the resistant pattern of *P. mirabilis* and *P. vulgaris* to the various antibiotics. The resistance plasmids against the various isolates were very diverse and distributive among the *Proteus* species (Table 2). These plasmids were also highly transferable with a high frequency range of $2x10^{-4}$ to $4x10^{-2}$. The plasmids bands of the strains ranged from ≤0.45kb to ≥1.25kb (Table 3). Most of the isolates were highly mediated by chromosomal resistant (Table 4). Forty four percent of the antibiotics were plasmids mediated, 32% by chromosome while 24% of the resistant pattern to the antibiotics could not be ascertained. The results indicate that the plasmids were able to move genetic antibiotics resistance materials among the various bacterial strains.

The rapid emerging of antibiotic resistant transfer genes among bacterial population are becoming important causes of clinical infections. These bacterial resistant genes are more vulnerable in debilitated patients especially those with immunosuppressed complications such

Table 1. Occurrence of resistant pathogens from diabetic wounds of diabetes percentage of resistant isolates to antibiotics.

Isolates	No. of isolates	Am	Ce	Co	N	Na	Cip	Nb	Pef	Gn	C
P. mirabilis	97	39 (40.2%)	6 (6.2%)	25 (25.8%)	13 (13.4%)	17 (17.5%)	5 (5.2%)	19 (19.6%)	4 (4.1%)	16 (16.5%)	21 (21.6%)
P. vulgaris	51	21 (41.2%)	9 (17.6%)	13 (33.3%)	15 (29.4%)	12 (23.5%)	4 (7.8%)	9 (17.6%)	3 (5.9%)	10 (19.6%)	17 (33.3%)
Total	148	60 (40.5%)	15 (10.1%)	38 (25.7%)	38 (25.7%)	29 (19.6%)	9 (6.1%)	28 (18.9%	7 (4.7%)	26 (17.6%)	38 (25.7%)

The values in bracket represent percentage.
Am = Ampicillin, Cip = Ciprofloxacin, Gn= Gentamicin, Nb = Norfloxacin, C= Chloramphenicol, Na = Nalidixic acid, Co = Cotrimoxazole, Ce = Cefotaxime, N= Nitrofurantoin, Pef = Pefloxacin.

Table 2. Detection of the frequency of transfer of *Proteus* species resistant transconjugants markers.

Code	Resistant Markers of donors (DN)	EJRif^r+ DN	Inference	Frequency of transfer	Resistant marker of transconjugants
M19	CoNbGnNaCAmN	Growth	Significant	0.22×10^{-3}	Co-N-Am-C
M3	CoPefNbGnCipNaCAmN	Growth	Significant	0.13×10^{-1}	Gn-N-C
M4	NaAmNCo	Growth	Significant	0.25×10^{-1}	Am-Na-N
V5	NbCeCo	Growth	Significant	0.23×10^{-1}	Nb-C-Am
M18	NaAmNCo	Growth	Significant	0.23×10^{-2}	Am-Nb-Co
V9	CoGnCipNaCAmNPefNb	Growth	Significant	0.23×10^{-1}	Gn-Na-Am-N
V20	NaAmNCoCipCe	Growth	Significant	0.26×10^{-3}	Na-Co-N
V22	NNbGnCipNaCAmPef	Growth	Significant	0.24×10^{-1}	N-Gn-Am-Nb
M23	GnNaAmNNbCip	Growth	Significant	0.11×10^{-1}	Gn-Na-Nb
V15	CoGnCipNaAmNNb	Growth	Significant	0.24×10^{-2}	Am-Gn-N

DN = Donor strains of *Proteus*, EJRif^r = *E coli* rifampicin resistant sucrose negative recipient, M = *Proteus mirabilis* strains and V = *Proteus vulgaris* strains.
Am = Ampicillin, Cip = Ciprofloxacin, Gn= Gentamicin, Nb = Norfloxacin, C= Chloramphenicol, Na = Nalidixic acid, Co = Cotrimoxazole, Ce = Cefotaxime, N= Nitrofurantoin, Pef = Pefloxacin.

Table 3. Antibiotics resistant plasmid profiles of *Proteus* species transconjugants.

Code of isolates	Resistant Marker spectrum of donors (DN)	Plasmids profile (kb) of DN	Plasmids transfer(kb) transconjugants	Resistant marker Spectrum of transconjugants
TM19	CoNbGnNaCAmN	1.06, 0.77	1.06, 0.77	Co-N-Am-C
TM3	CoPefNbGnCipNaCAmN	1.25, 0.87	1.25, 0.87	Gn-N-C
TM4	NaAmNCo	1.25, 0.87	1.25	Am-Na-N
TV5	NbCeCo	1.25, 0.87, 0.45	1.25	Nb-C
TM18	NaAmNCo	1.25, 0.87	1.25, 0.87	Am-Nb-Co
TV9	CoGnCipNaCAmNPefNb	1.06, 0.77	1.06	Gn-Na-Am-N
TV20	NaAmNCoCipCe	1.25, 0.87	1.25, 0.87	Na-Co-N
TV22	NNbGnCipNaCAmPef	1.25, 0.87,0.45	1.25, 0.87	N-Gn-Am-Nb
TM23	GnNaAmNNbCip	1.25, 0.87,0.45	1.25, 0.87	Gn-Na-Nb
TV15	CoGnCipNaAmNNb	1.06, 0.77	1.06, 0.77	Am-Co-N

TM = *Proteus mirabilis* transconjugants, DN = donor strains, TV = *Proteus vulgaris* transconjugants.
Am = Ampicillin, Cip = Ciprofloxacin, Gn= Gentamicin, Nb = Norfloxacin, C= Chloramphenicol, Na = Nalidixic acid, Co = Cotrimoxazole, Ce = Cefotaxime, N= Nitrofurantoin, Pef = Pefloxacin.

Table 4. Chromosomally mediated antibiotic resistant markers of *Proteus* species.

Code of isolates	Resistant spectrum before curing (wthout SDS)	Resistant spectrum after curing (treatment wth SDS)
CM19	CoGnNaCAmN	Na-C-Am-Gn
CM3	CoGnNaCAmN	-
CM4	NaAmNCo	Co-Am-N
CV5	NbCeCo	Nb-Co
CM8	GnCCo	C-Gn
CV9	CoGnCipNaCAmNPefNb	Co-Pef-Na
CV10	NNbCoNaCAm	Nb-Co-Na-C
CV20	NaAmNCoCipCe	Cip
CM22	NNbGnCipNaCAmPef	C
CV15	CoGnCipNaAmNNb	N-Nb
CV18	NaAmN	-

CM = Chromosomally cured resistant *Proteus mirabilis,* CV = chromosomally cured resistant *Proteus vulgaris*, SDS = Sodium dodecyl sulfate.
Am = Ampicillin, Cip = Ciprofloxacin, Gn= Gentamicin, Nb = Norfloxacin, C= Chloramphenicol, Na = Nalidixic acid, Co = Cotrimoxazole, Ce = Cefotaxime, N= Nitrofurantoin, Pef = Pefloxacin.

as HIV/AIDS, cancers, diabetic wounds, burns, etc (Frier et al., 1999; Prescott et al., 2001). It is often very important to be able to compare strains of different species obtained from these patients as this may be very useful in epidemiological studies especially when disease outbreak has occurred (Prescott et al., 2001). In the current study, antibiotics resistant profiles of *Proteus* species from diabetic wounds were studied. The results indicated that *Proteus* species harbors a wide range of varied molecular weights antibiotic resistant plasmids (≤ 23.1 kb). Out of a total 148 *Proteus* species, 76 (51.4%) had varied multi-drug resistant markers, of which some are encoded on transferable plasmids. The frequency of transfer of the resistant plasmids ranged from $2x10^{-4}$ to $4x10^{-2}$ per donor cells. It was therefore evident that antibiotics resistant plasmids markers among *Proteus* species could easily be transferred by conjugation. This is because the resistant patterns of the transconjugants were similar to the resistant pattern of the donor cells.

The positive transconjugant electrophoretic pattern also revealed plasmids showing approximate relative distances, sizes and molecular weights similar to their respective parent donor cells (≤ 23.1kb). This high frequency of transfer coincided with those of Yukata et al. (2004) that ranged from $2x10^{-4}$ to $6x10^{-1}$ per donor cells while characterizing conjugation experiment of *E. coli* producing CTX-M-2 β-lactamase in Japanese cattle. Also Wang et al. (2004) while detecting plasmids-mediated quino-

lones resistant markers of *Klebsiella pneumoniae* in the United States selected seven quinolones positive donor cells with sulfamethoxazole at a high frequency of $2x10^{-4}$ to $8x10^{-4}$ per donor cells. The present study also showed that *Proteus* species harbors highly transferable resistant plasmids markers to ampicillin, followed by nitrofurantoin, gentamicin, nalidixic acid and norfloxacin. None of the *Proteus* species had plasmids resistant transferred markers of ciprofloxacin, pefloxacin and cefotaxime.

In the studies of Yukata et al. (2004), all the isolates were able to transfer cefotaxime resistance to the recipient cell which deviated from the current studies where some of the species lack discernible resistant transfer markers. In the work of Wang et al. (2004) all the isolates were resistant to ampicillin and were encoded on transferable plasmids as well as in their transconjugants. These transferable resistance markers between different bacterial isolates may go unnoticed by infection control methods, therefore undermining hospital infections control policies (Nashwan et al., 2005). The result also indicated that there was no correlation between the number of antibiotics that a plasmid mediates to and its size. Although other findings have reported plasmids mediated resistance with similar genetic backbone sharing similar resistant profiles (Miranda et al., 2004). In the current study, plasmids with the same relative distance on electrophoresis, size and molecular weights had varied antibiotic resistance patterns.

Apart from *Proteus* species, widespread drug resistance plasmids mediated genes have been widely reported in *E. coli, Salmonella, Shigella, Klebsiella* and *Staphylococcus* (Motta et al., 2003; Po-Ren et al., 2004). Although it has been reported that most plasmids acquire their antibiotic resistance genes through transposons, chromosomes or from other plasmids (Norma et al., 2004), it is necessary to elaborate and enhance ways of controlling these phenomenon.

The current study also shows that the *in vitro* analysis challenging transfer experiments was the inability of plasmids reception by the standard strain and the rate at which the plasmids are lost during samples analysis. It was observed that successive sub-cultures of the *Proteus* species resulted in rapid lost of the plasmids than from freshly analyzed diabetic wound samples. The incidence of plasmids was also higher in freshly cultured diabetic wound sample isolates than in old stored cultured diabetic wound samples.

From the curing experiments, sodium dodecyl sulfate (SDS) was found to eliminate plasmids from the bacteria cells. The inability of some of the *Proteus* species to exhibit particular resistant pattern after curing indicated that resistance to some antibiotics were plasmids or chromosome mediated or both plasmids and chromosome mediated. Enabulele et al. (1993) reported that 13.6% of gram-negative bacteria isolated from infected wounds from the University Benin Teaching Hospital were plasmids mediated after curing. Plasmids can also be lost through successive sub-cultures (Prescott et al., 2003). Ethidium bromide has also been reported as a good curing reagent of plasmids from cells. After curing, the cells were lysed and separated electrophoretically as previously described by Meyers et al. (1976). Most of the separated electrophoretic DNA bands had only the chromosomal molecular weight > 23.1kb without/or with large plasmids DNA bands.

In developing countries, however, antibiotic resistance is highly linked to inappropriate use of antibiotics, lack of health care personnel with continual health education on antibiotics and poor quality drugs. Therefore, continual surveillance of antibiotic resistance in developing countries is important to alleviate morbidity and mortality rate of various microbial infections.

The sizes of the plasmids varied and resistance markers showed that there was no plasmid epidemic involved in the antibiotic resistance against *Proteus* species. Unfortunately, resistance among enterobacteria isolates has become increasingly common, making empirical treatment more difficult based on antibiotic resistant plasmids. *Proteus* species are being isolated at an increasing rate in hospital settings and are having a significant impact on clinical practice and treatment cost. According to Miranda et al. (2004), these clinically derived plasmids do not belong to distinct plasmid lineages but exhibit evidence of broad scale inter-plasmid gene transfer probably involving a range of mechanisms such as recombination, transposition and integration.

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 and Rauol Ndasi of the Department of Histopathology ABUTH Zaria for their assistance.

REFERENCES

Adel A, Zouyheir IB, Abdullah AS, Lubna AM (2005). Bacteriological study of diabetic foot infections. J. Diabetes Complicat. 19(3): 138-141.

Asad UK, Amna M (2004). Plasmid-mediated multiple antibiotic resistance in *Proteus mirabilis* isolated from patients with urinary infection. Med Sci. Monit. 10(1): CR598-602.

Birnboim HC, Doly J (1979). A rapid alkaline extraction procedure for screening recombinant DNA plasmids, Nucleic Acids Res., 7: 1513-1523.

Cheesbrough M (2000). District Laboratory Practice Manual in Tropical Countries. Part 2. Cambridge University Press. pp. 178-179.

Cowan ST, Steel KJ (1993). Manual for the identification of medical bacteria. Cambridge University press. London, New York, Rockville, Melburne and Sydney.

Enabulele IO, Ogbimi AO, Obuekwe CO (1993) Incidence of plasmids in Gram-negative bacterial isolates from infected wounds. Niger. J. Microbiol. 9: 13-16.

Enabulele IO, Yah SC, Yusuf EO, Eghafona NO (2006). Emerging quinolones resistant transfer genes among gram-negative bacteria, isolated from faeces of HIV/AIDS patients attending some Clinics and Hospitals in the City of Benin, Edo State, Nigeria. Online J. Health

Allied Scs. 3: 3.
Frier BM, Truswell AS, Shepherd J, Loog de A, Jung R (1999). Diabetes mellitus and nutrition and metabolic disorders in: Davidson's principles and practice of medicine 8th Edition, Christopher H, Edwin RC, John AH, Nicholas AB, Churchill, Livingstone, London, Edinburgh, New York, Sydney. pp. 471-542.
Goldstein EJC, Citron DM, Nesbitt CA (1996). Diabetic foot infection, Diabetes Care 19: 638-641.
Jun IW, Kunikazu Y, Keigo S, Hiroshi K, Naohiro S, Satowa S, Yohei, D, Kouji, K, Yasuyoshi I, Yoshichika A (2006). Novel plasmid-mediated 16S rRNA methylase, RmtC, found in a *Proteus mirabilis* isolate demonstrating extraordinary high-level resistance against various aminoglycosides. Antimicrob Agents Chemother. 50: 178-184.
Kumar P, Clark M (2002). Diabetes Mellitus and other Disorders of metabolism, Clinical Medicine. W.B. Saunders Press Ltd. London. pp.1069-1100.
Louie TJ, Barlett JG, Tally FP, Gorbach SL (1976). Aerobic and anaerobic bacteria in diabetic foot ulcers. Ann. Int. Med. 85: 461-463.
Meyers JA, Sanchez D, Elwell LP, Falkows S (1976). Simple Agarose gel electrophoretic method for the identification and characterization of plasmids deoxyribonucleic acid. J. Bacteriol. 127: 1529-1537.
Miranda S, David MG, Peter JC (2004). Evolution of multi-resistance plasmids in Australia clinical isolates of *Escherichia coli.* Microbiology 150: 1539-1549.
Motta RN, Oliveira MN, Magalhaes PSF, Dias AM, Aragho LP, Forti AC, Carvalho CBM (2003). Plasmid-mediated extended spectrum β-lactamase producing strains of enterobacteriaceae isolated from diabetes foot infections in Brazilian Diabetic Center. Braz. J. Infect. Dis. 7(2): 129-134.
Nashwan AIN, Brighta D, Paul HMS, Loddewijk S, Evert de Jonge, Aldert, B, Christina MVG, Menno DDJ (2005). +Widespread transfer of resistance genes between bacterial specie in an intensive care unit: Implication for Hospital Epidemiology. J. Clin. Microbiol. 43(9): 4862-4864.
National Committee for clinical laboratory standards (2000). Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow aerobically, 4th Edition, Approved Standard M7-A4.NCCLS, Vilanova, P. A.
Nelson P (2006). Diabetes mellitus. J. Appl. Sci. Environ. Manage. 1: 1-2.
Norma SL, Anita T, Dailia PR, Eliane MFR, Bianca RQ, Ernesto H (2004). Antimicrobial resistance and R-plasmid in Salmonella spp. From swine and abatoir5 environment. Pesq Vet Bres. 24(2): 57-60.
Olukoya DO, Oni O (1990). Plasmids profile analysis and antimicrobial susceptibility patterns of Shigella isolates from *Nigeria*. Epidemiol. Infect. 105: 59-64.
Po-Ren H, Lee JT, Sung PT, Chao FC, Jen HW, Jing JY, Chun ML, Yin CC, Wen KH, Cheng YL, Shen WH, Kwen TL (2004). Ciprofloxacin-resistant *Salmonella enterica typhimurium* and *S choleraesuis* from pigs to Humans, Taiwan.. *Emerging Infectious* Diseases www.cdc.gov/veid 10(1): 60-66.
Prescott LM, Hardy MP, Klein JP (2001). Microbiology. 4TH Edition McGraw Hill New York.
Ravisekhar G, Benu D, Vishnubhatla S, Arti K, Ammini AC, Rama C (2006). Clinical microbiological study of diabetic foot ulcers in an Indian tertiary care hospital, Diabetic Care. 29: 1727-1732.
Unachukwu CN, Obunge OK, Odia OJ (2005). The bacteriological of Diabetic ulcers in port Harcourt, Nigeria. Niger. J. Med. 14(2): 173-176.
Wang M, Sahm MF, Jacoby GA, Hooper DC (2004). Emerging plasmid-mediated quinolone resistance associated with the qnr gene in *Klebsiella pneumoniae* clinical isolates in the United States. Antimicrob. Agents Chemother. 48(4): 1295-1299.
Yah SC, Enabulele IO, Eghafona NO (2004). Bacteriological studies on infected Kerosene burn wounds in Benin City, Nigeria. J. Biomed. Invest. (JBI)., 2(1): 4-9.
Yukata S, Naohiro S, Yohei D, Yosshichika A (2004). *Escherichia coli* producing CTX-M-2 ß- Lactamase in cattle , Japan. Emerg. Infect. Dis. www.cdc.gov/eid. 10(1): 69-75.
Zhou C, Yang Y, Jong AY (1990). Using mini plasmids DNA for sequencing double stranded template with sequenase. Biotechniques, 8: 172-173.