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Full Length Research Paper

Uropathogens isolated from HIV-infected patients from Limpopo Province, South Africa

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The primary aim of this study was to determine the prevalence and antibiotic susceptibility profiles of uropathogens isolated from HIV-infected patients in Limpopo Province, South Africa. One hundred and ninety-five urine samples were obtained from HIV-infected patients between June 2008 and May 2009 and the samples were investigated using standard and conventional microbiological methods. Urinary tract infections (UTIs) were detected in 95 samples. *Enterobacter* species (37.6%) was the most prevalent uropathogen. Other bacterial isolates included *Escherichia coli* and *Klebsiella* species, each constituting 17.9%, *Citrobacter* species (9.7%), *Proteus* species (7.4%) and others (11.6%). Resistance to trimethoprim/sulfamethoxazole (SXT) which is used for empiric therapy was above 35%. However, majority of the isolates were susceptible to amikacin, ciprofloxacin and most of the second generation cephalosporins as well as imipenem. Resistance of uropathogens to SXT in Limpopo Province is higher than the recommended IDSA setting and this requires the introduction of an alternative first-line therapy.

Key words: Antibiotic resistance, HIV, uropathogens, urinary tract infections.

INTRODUCTION

Acute urinary tract infections (UTIs) remain one of the most common problems for which patients seek medical treatment in the community (Mazzulli et al., 2001). Women are significantly more likely to experience UTIs than men as nearly 1 in 3 women will have had at least 1 episode of UTI requiring antimicrobial therapy by the age of 24 years (Foxman, 1990). Almost half of all women will experience 1 UTI during their lifetime. Specific subpopulations at increased risk of UTIs include infants, pregnant women, the elderly, and patients with diabetes, multiple sclerosis, patients with acquired immunodeficiency disease syndrome (AIDS) or human immunodeficiency virus (HIV) infected individuals and patients with underlying urological abnormalities.

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More than 90% of UTIs are due to enteric Gram negative organisms, of which *Escherichia coli*, *Enterobacter, Proteus* and *Klebsiella* are commonly implicated. Current management guidelines recommend empirical therapy for acute, uncomplicated lower UTIs in young women (Foxman, 1990; Stamm, 1992; Zhanel et al., 1998; Mazzulli et al., 2001; Sahm et al., 2001; Deokar and Bodhanker, 2009). Almost all the causative organisms of UTIs originate from fecal materials or the periurithral environment (Muratani and Matsumoto, 2004). UTIs accounts for a large proportion of antibacterial drug consumption (Olson et al., 2009). Because majority of the treatments are done empirically, it is necessary to have a good knowledge of the causative agents, their epidemiological characteristics and their antibacterial susceptibility profiles.

These data will be of great value and imperative for optimizing treatment and reduction of treatment failures,

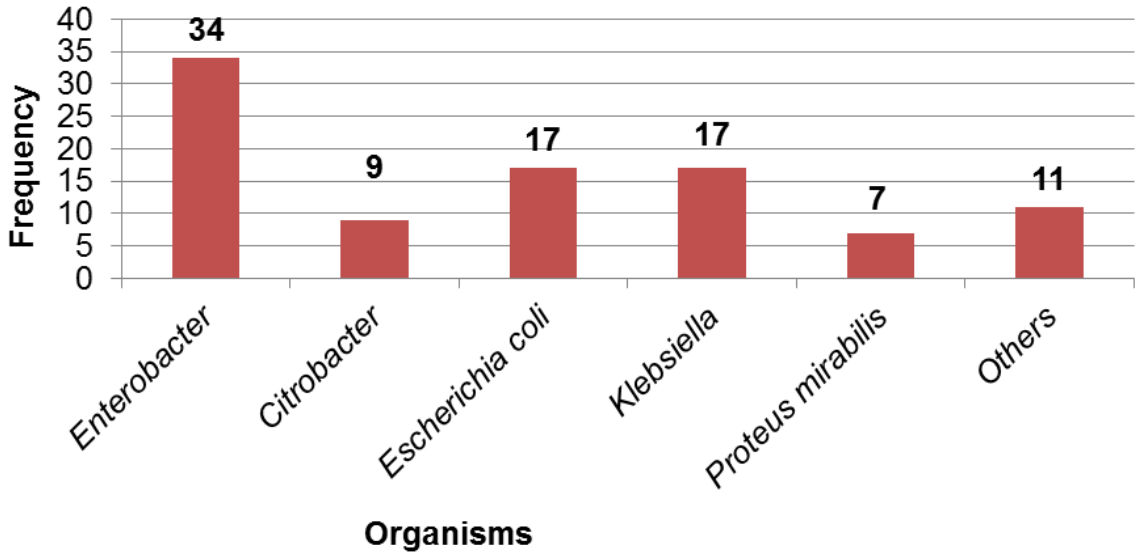

Figure 1. Prevalence of uropathogens isolated from HIV-infected patients.

which may be due to the emergence of antibacterial resistance (Adedeji and Abdulkadir, 2009).

Urinary tract infections are among one of the most common bacterial infections, and the cause of morbidity and hospitalization in HIV positive individuals. Bacterial infections are common among HIV infected patients. However, prevalence of data on the frequency of UTIs in HIV-infected patients are limited, even though some studies showed that community acquired UTIs are more common among them as compared to control groups, and that the incidence rate of nosocomial UTIs is 1.2 per 1000 patient-days (Petrosillo et al., 2002). A decrease in CD4+ count is at least partially responsible for the profound immunodeficiency that leads to various opportunistic infections in the HIV-infected persons.

The aim of this study was to determine the prevalence and antibiotic susceptibility profiles of isolated uropathogens from HIV-infected individuals in Limpopo Province of South Africa where no previous data on UTIs in HIV-infected patients exist.

MATERIALS AND METHODS

Between July 2008 and May 2009, 195 urine samples were collected from HIV positive individuals in Limpopo Province in South Africa and were investigated for the presence of pathogens. Study participants included 150 females and 45 males, all of which were 18 years and above. Samples were collected from patients who were not on antibiotic treatment, while those that have had antibiotic treatment 2 months prior to sample collection were excluded. Samples were collected after oral informed consent was given by the patients.

Specimen collection and processing

A total of 195 urine samples were collected from the study participants. These samples were the first “clean catch” collected in sterile clean bottles. The samples were immediately transported to the lab in an ice cooler box. Culture was done and the isolates were identified using standard microbiological techniques. 0.5 ml of each sample was inoculated into MacConkey agar and CLED (cysteine-lactose electrolyte deficient agar) media, respectively and incubated for 24 h at 37°C. The numbers of colony forming units (cfu/ml) was counted and samples with $>10^5$ cfu/ml were considered positive. Isolates were identified on negative combo plates using the MicroScan auto SCAN4. The plate which has 96 wells contains wells for biochemical tests for isolates identification as well as antibiotics for sensitivity profiling according to Clinical and Laboratory Standards Institute (CLSI) recommendation. Antibiotics included in the panel were: amikacin, amoxicillin/clavulanate, ampicillin, aztreonam, cefazolin, cafepime, cefotaxime, cefotaxime/clavulanate, cefoxitin, ceftazidime, ceftazidime/ clavulanate, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, piperracillin, pip/tazo, tetracycline, ticar/k clavulanate, tobramycin and trimethoprim/sulfamethoxazole.

RESULTS

In total, 195 HIV-1 positive patients were included in this study. The age of the patients ranged from 18 to 56 years, with mean and median ages of 37 years. Uropathogens were detected in 95 samples (48.7%) of which 85 were from females. The 95 urinary tract isolates from the patients included 34 (37.6%) of *Enterobacter* spp. isolates, 17 (17.92%) each of *E. coli* and *Klebsiella* spp. 9 (9.7%) of *Citrobacter* isolates, 7 (7.4%) of *Proteus* spp. and 11 isolates of other organisms including one isolate each of *Serratia fonticola*, *Providencia rettgeri, Pseudomonas stutzeri* and *Morganella morganii*, while the 5 (5.3%) *Staphylococcus* spp. and 1 isolate of *Listeria monocytogenes* were the only Gram positive bacteria that were isolated (Figure 1).

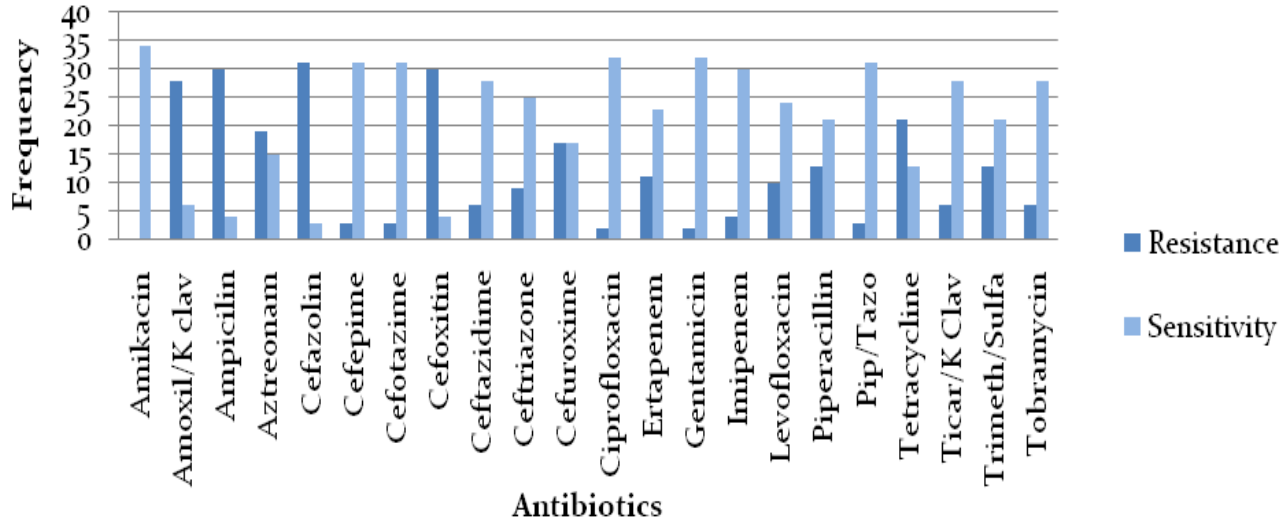

Figure 2. Antibiotic sensitivity profile of *Enterobacter* spp. from the studied samples.

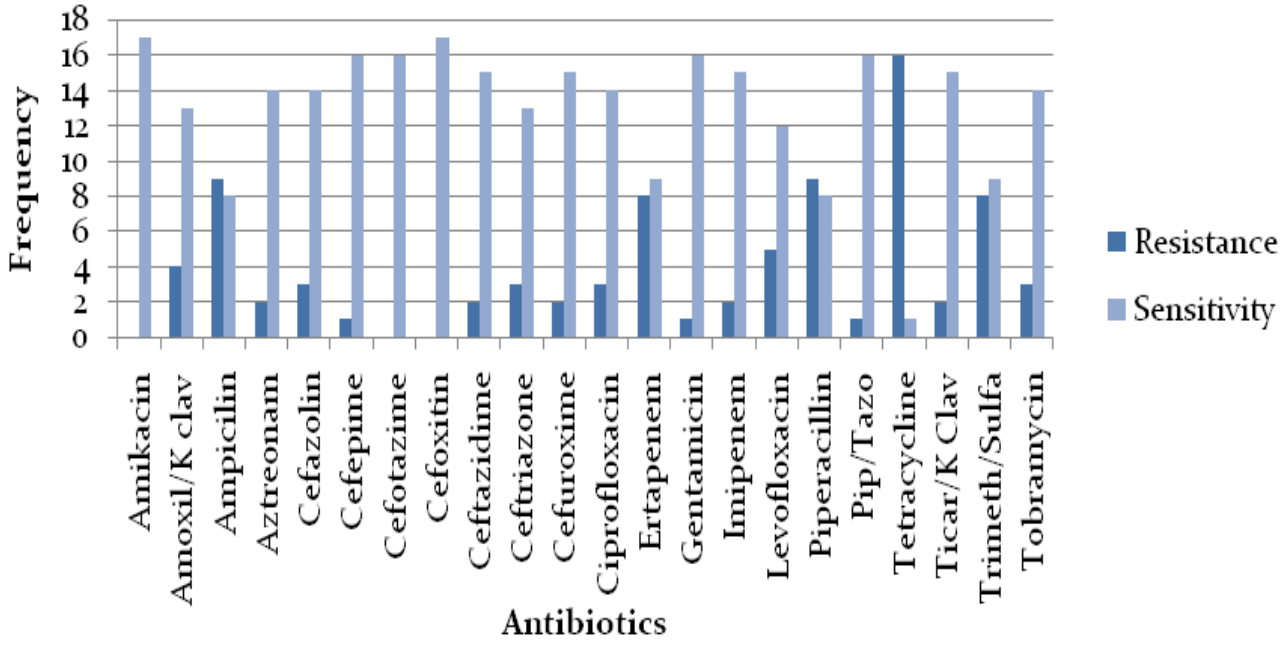

Figure 3. Sensitivity pattern of *E. coli* isolated from HIV-infected patients.

Antibiotic sensitivity profiles of the isolates showed all 34 *Enterobacter* isolates to be sensitive to amikacin, while over 45.3% of the isolates were resistant to amoxil/clavulanate, ampicillin, cefazolin, cefoxitin, entrapenem, piperacillin, tetracycline and SXT. However, sensitivity was shown by a greater number to cefepime, cefotazidime, ciprofloxacine, gentamicin, imipenem, piperacillin/tazocin, ticarcillin/k clavulanate and tobramycin with sensitivity greater than 85% as shown in Figure 2.

The 17 *E. coli* isolates were all sensitive to amikacin, cefotazime, cefoxitin, while resistance was more than 23% to amoxil/clavulanate, ampicillin, entrapenem, levofloxacin, ciprofloxacin, piperacillin, tetracycline and SXT, respectively. Sensitivity to aztreonam, cefazolin, cefepime, ceftazidime, cefuroxime, imipenem, piperacillin/tazobactam and tobramycin was above 80% in each case as shown in Figure 3.

Similarly, all the *Klebsiella* isolates were sensitive to amikacin and imipenem. Sensitivity was also above 80% among the isolates to ceftazidime, ciprofloxacin, gentamicin, pip/tazo, ticar/k clavulanate, SXT and tobramycin while all the isolates were resistant to ampicillin, 82.5% to cefazolin, 9 4% to piperacillin, while

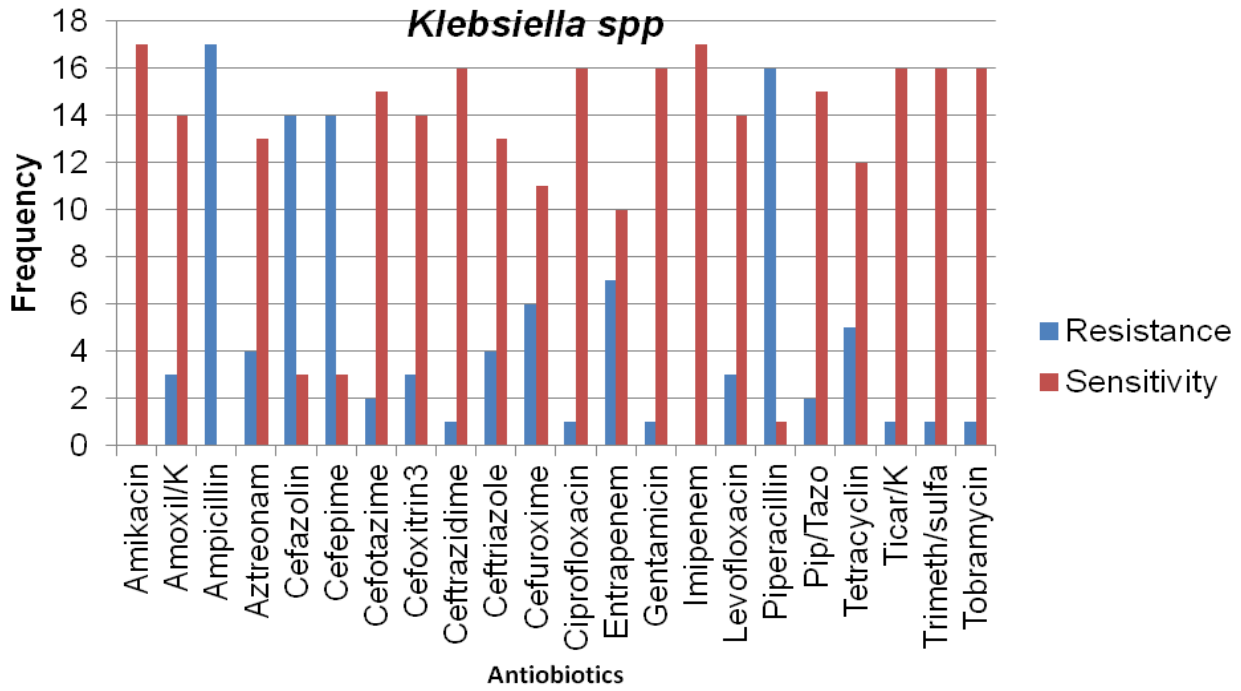

Figure 4. Sensitivity pattern of *Klebsiella* spp. isolated from HIV-infected patients from Limpopo Province, South Africa.

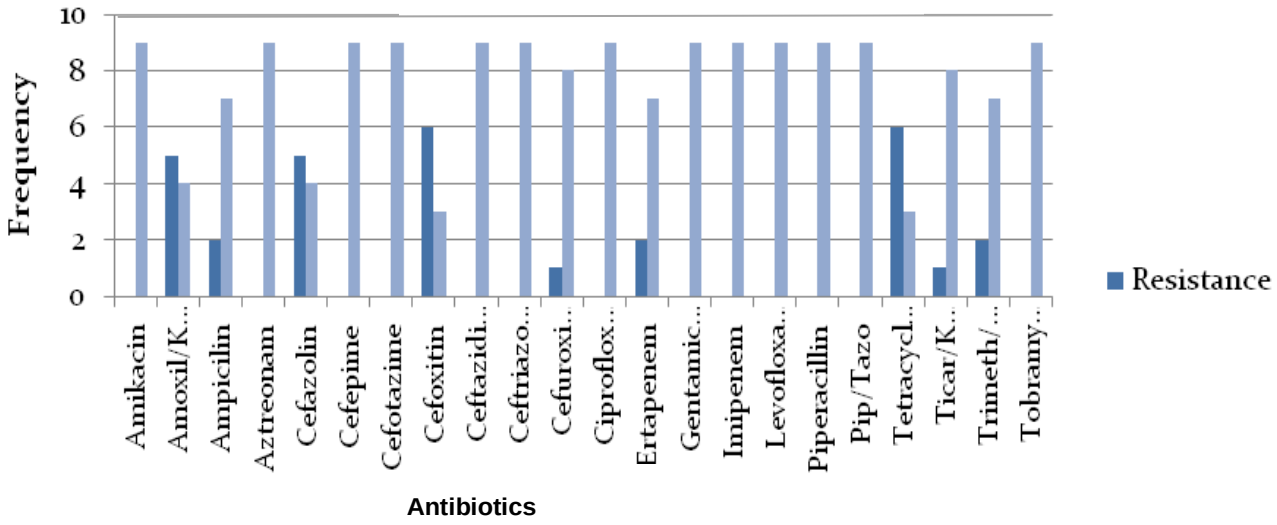

Figure 5. Sensitivity pattern of *Citrobacter freundii* complex isolates from Limpopo Province, South Africa.

17.6% were resistant to amoxil/clavulanate, aztreonam, cefeprime, cefoxitin, ceftriazone, cefuroxime, levofloxacin and tetracycline, respectively, as shown in Figure 4.

Citrobacter spp. exhibited the greatest sensitivity among the isolates as they were sensitive to majority of the classes of drugs that were tested against them. They showed 100% sensitivity to amikacin, aztreonem, cefepime, cefotazidime, ceftriazone, ciprofloxacin, gentamicine, imipenem, levofloxacin, piperacillin, piperacillin/tazocin and tobramycin. Resistance to tetracycline and cefoxitin was 66%, while resistance to amoxil/clavulanate and cefazolin was 55.5%, respectively. Sensitivity to ampicillin, cefuroxime, entrapenem, ticarcillin/ k clavulanate and SXT was however above 78% as shown in Figure 5.

All the *Proteus* spp. exhibited some degree of resistance to all the antibiotics in the panel as seen in Figure 6.

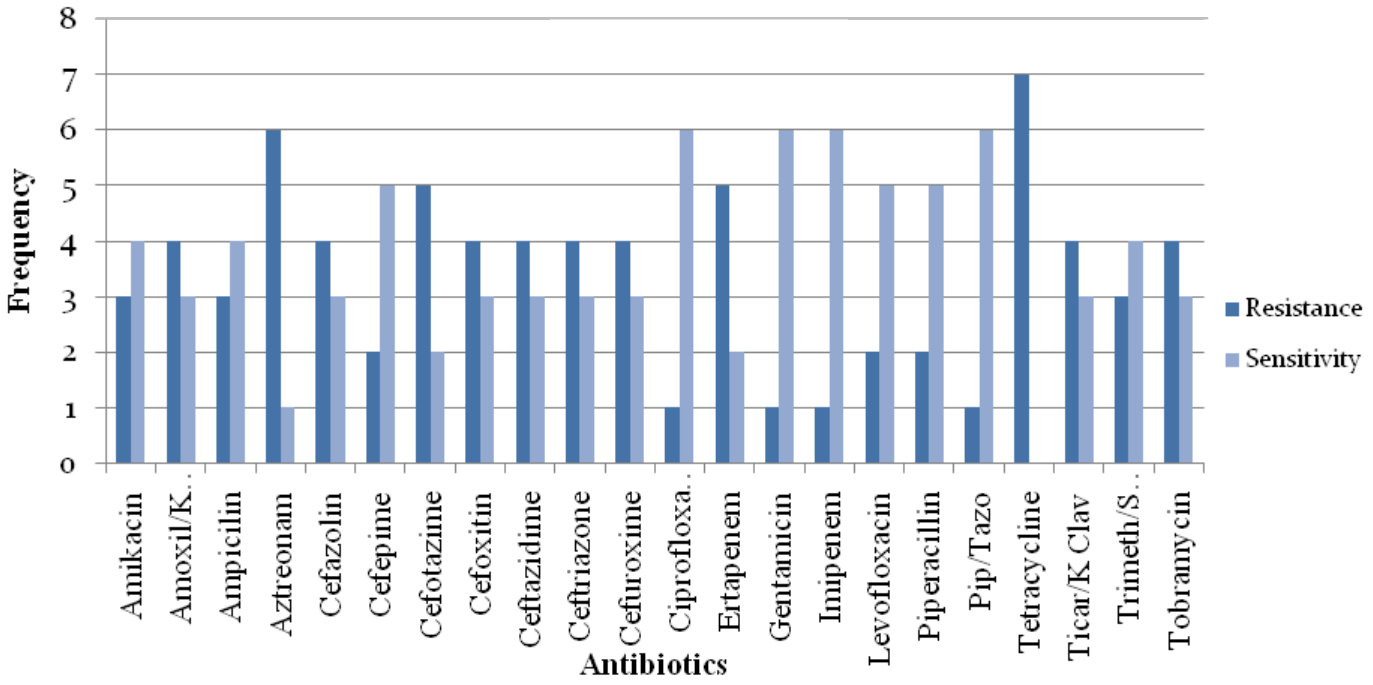

Figure 6. Sensitivity pattern of *Klebsiella* spp. isolated from HIV-infected patients from Limpopo Province, South Africa.

DISCUSSION

In this study, we found that the prevalence of UTIs was significantly higher in female (89.5%) than in male HIV-1 positive patients. Evidence from various epidemiological studies showed that UTIs were more common in female than in their male counterparts (Foxman, 1990; Hasan et al., 2007; Buzayan and Taher, 2009). The high level of infection in the urinary tract of HIV- infected women may be determined by the number of microorganisms located in the vagina (Wheat, 1980). UTIs appears to be multi-factorial in subjects with HIV infection as the WHO stage of the disease, the CD4 level, and other complications of the urinary tract are significantly associated with UTI among subjects with HIV infection. Omoregie and Eghafona (2009) reported a correlation between HIV infection and urinary tract infections as there was a significant difference in the prevalence of asymptomatic UTI between HIV patients and non-HIV subjects.

Bacteriological studies usually reveal the involvement of Gram negative enteric organisms such as *E. coli*, *Klebsiella* spp., *Proteus* spp. as the common cause of UTIs (Hasan et al., 2007; Kose et al., 2007; Deokar and Bodhankar, 2009; Ramesh et al., 2008). Similarly, in our study, the predominant numbers of pathogens isolated were Gram negative bacilli of the family enterobacteriaceae rather than Gram positive pathogens. In almost all studies, members of enterobacteriace are usually implicated with preponderance of either *E. coli* or *Klebsiella* spp. Zhanel et al. (1998) reported the predominance of *E. coli* from UTIs isolates in Canada. Similarly, Hasan et al. (2007), Janifer et al. (2009) Adedeji and Abdulkadir (2009) and Kose et al. (2007) reported the predominance of *E. coli* among uropathogens isolated from different locales, with *Enterobacter* in less significant number. In this study, we reported the preponderance of *Enterobacter* spp. with a prevalence of 35.8% and *E. coli* and *Klebsiella* spp. each constituting 17.9%, were noted to vary markedly from those noted by Petrosillo et al. (2002) and De Pinho et al. (1994) that found *E. coli* to be predominant pathogen associated with UTI. Pead et al. (2006) reported 30 and 20% of UTI cases due to *Klebsiella* species and *S. aureus*, respectively.

Antibiotic resistance has become a consideration in the treatment of community-acquired UTIs (Nicolle, 2002). Resistance to several antimicrobial agents was prevalent among the isolates recovered from the study participants. In about three decades ago, multidrug resistance (MDR) was practically non-existent and the cause was restricted to mutations of chromosomal genes (Hasan et al., 2007). However, in the last decade, bacterial resistance mediated by plasmids which carry resistance genes to a large number of antibiotics that are rapidly transferred has worsened the situation (Ram et al., 2000).

In their study from South India, Ram et al. (2000) reported that anti-microbial resistant genes clustered in integrons. According to Mathail et al. (2004), resistance to ampicillin, cotrimaxazole, trimethoprim, nalidixic acid, chloramphenicol, tetracycline and gentamicin are common in isolates with integrons. Multidrug resistance was common among all the genera isolated in this study.

Particularly, resistance was higher than 45% among the isolates to amoxil/k clavulanate, ampicillin, tetracycline, trimethoprime-sulfamethoxazole, piperacillin, entrapenem and cefaxolin. Ampicillin and trimethoprim-sulfamethoxazole (SXT) resistance among urinary tract isolates has been reported with increasing frequencies (Gupta et al., 1999; Hooton and Stamm, 1997; Zhanel et al., 1998).

Our results suggest that most community Gram-negative urinary tract isolates remain susceptible to amikacin, tobramycin, piperacillin,/tazocin, tycarcillin, clavulanate, imipenem, gentamicine and to most of the second generation cephalosporins such as cefepime, cefotazime, ceftrazidime, ceftrizone, as well as ciprofloxacin.

To our knowledge, there is no previous study on antibiotic resistance among uropathogenic isolates from HIV-infected persons from Limpopo Province. In Europe, *E. coli* susceptibility for multiple drugs varied from 9 to 40% in uncomplicated UTIs in women, resistance to fluoroquinolones varied from 9 to 16% (Hummers-Pradier et al., 2005; Mahamat et al., 2005). We reported in this study, the prevalence of 12.6% resistance of the isolates to ciprofloxacin and 29.5% resistance to levofloxacin. *Proetus* spp. had a maximum overall resistance as 57% of the isolates showed a high degree of resistance to all the antimicrobial agents that were tested against them. *Citrobacter freundii* complex exhibited the greatest sensitivity as 33.8% of the isolates showed resistance to the panel of antibiotics, followed by *Enterobacter* with 48.6%, while *E. coli* and *Klebsiella* spp. each showed 53% resistance to the antimicrobial agents. Given that the majority of therapy for UTIs is empiric and that uropathogens are demonstrating increasing antimicrobial resistance, it is our recommendation that antibiotic resistance profiling of uropathogens be carried out prior to commencement of therapy. The Infectious Disease Society of America (IDSA) guidelines suggest that in community with SXT resistance rates of ≥10 to 20% among UTI pathogens, alternative antimicrobial agents should be considered as first-line treatment for acute uncomplicated bacterial cystitis in women (Olson et al., 2009). Incumbent on these recommendations is the need to perform surveillance to ensure that activities of SXT and alternative agents are maintained and that emerging resistance trends, such as MDR are identified (Sahm et al., 2001).

In conclusion, in HIV–infected patients with culture-positive UTIs in Limpopo Province, resistance to SXT has exceeded 35%, so this agent should no longer be used for empirical therapy. Amikacin, ciprofloxacin, imipenem, ticarcillin k clavulanate, piperacillin/tazocin as well as second generation cephalosporins may be appropriate for empirical therapy in the management of UTIs in HIV-infected patients in Limpopo Province. There were only three cases of resistance to amikacin by *Proteus mirabilis*, suggesting that the agent should be considered as a first-line empirical treatment for uncomplicated lower UTIs.

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