

## Prevalence and antimicrobial resistance in *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates from non-clinical urine samples.

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**Abstract:** A total of 120 urine samples from apparently healthy students were analysed for the prevalence of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The activity of 13 antimicrobials was also studied to determine antimicrobial resistance rates and multiple resistances amongst the isolates. Antimicrobial resistance test was carried out by the Kirby-Bauer technique. The *P. aeruginosa* isolates exhibited high resistance to streptomycin, sparfloxacin and ciprofloxacin (39-74%), and moderate resistance to ofloxacin, gentamicin and pefloxacin (19-35%). The *K. pneumoniae* isolates exhibited moderate resistance to augumentin, co-trimoxazole and amoxicillin (22-29%). *P. aeruginosa* was more prevalent (80%) in the samples than *K. pneumoniae* (55%). The isolates also occurred more with the female students than the males. Resistances to the antimicrobials for both isolates were equally higher in the females than in the males. The results probably infer a great measure of abuse (overuse or misuse) of antimicrobials among the student population, considering the fact that the urine samples were obtained from students who were neither on admission in the hospitals nor out patients for urinary tract infections.

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**Key words:** Prevalence, antimicrobial resistant, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, non-clinical, urine samples.

### Introduction

The tremendous therapeutic advantage afforded by antibiotics is being threatened by the emergence of increasingly resistant strains of microbes. Selective pressure favouring resistant strains arises from misuse and overuse of antimicrobials (File, 1999; Livermore, 2005). The world wide use of antimicrobials in different fields has created enormous pressure for the selection of resistance among opportunistic bacterial pathogens (Balostescu *et al.* 2003; Sharma *et al.* 2005).

According to Harberth *et al.* (2000), inadequate and prolonged antimicrobial prophylaxis increases resistance to antimicrobial drugs. Indeed this is more evident in developing countries like Nigeria, where drugs including antibiotics can be purchased over the counter without a doctor's prescription. In addition, antimicrobials are hawked and dispensed at street and market corners by any body that has the means. This of course comes with its concomitant abuse due to lack of proper legislation.

In recent years antibiotic resistance has become a significant human health issue. Multiple antimicrobial resistant bacteria are considered, presently as a great global threat to public health. Today we can list a number of organisms in both hospitals and the community that thwart treatment because they are resistant to not one, but o many different antibiotics (Levy, 1998).

The term multi drug resistance (MDR), which initially describes resistant mammalian tumour cells and later strains of *M. tuberculosis*, now describes multi drug resistance in any micro organism – bacterium, fungus or parasite. The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used (Levy, 1997).

Urinary tract infections (UTIs) are bacterial infections that affect any part of the urinary tract. Although urine contains a variety of fluids, salts and waste products, it does not usually have bacteria in it. When bacteria gets to the bladder or kidney and multiply in urine, they may cause a urinary tract infection. The symptoms include frequent feeling and / or need to urinate, pain during urination and cloudy urine. The risk factors for UTIs include sexual activity, gender, genetics and presence of urinary catheters (Nicolle, 2008). Women are more prone to UTIs than men because in females, the urethra is much shorter and much closer to the anus than in males. Females also lack the bacteriostatic properties of prostatic secretions (Nicolle, 2008).

Many groups of bacteria have been implicated as causative agents of UTIs and include *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp, *Pseudomonas aeruginosa*, *Proteus* sp and *Streptococcus* sp.

*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, both Gram-negative rods, are major

causes of nosocomial infections world wide. *Pseudomonas aeruginosa* is aerobic, found in water, soil, plants and humans. Its minimal nutritional requirements, tolerance to a wide variety of environmental conditions and relative resistance to antimicrobial agents contribute to its ecological success and to its role as effective opportunistic pathogens (Gales *et al.* 2001). In *P. aeruginosa*, antimicrobial resistance may arise because of outer membrane impermeability, increased activity of multidrug efflux pumps, target site alterations or enzymatic degradation (Karlowsky *et al.* 2003).

Hospital reservoirs of the micro organism include respiratory equipments, antiseptics, soaps, sinks, mops, hot tubs, artificial finger nails, physiotherapy and hydrotherapy pools (Giamarellou, 2002). According to Sader *et al.* (2001), *Pseudomonas aeruginosa* is primarily a nosocomial pathogen and it rarely affects healthy persons. In recent times, the emergence of MDR *P. aeruginosa* strains resistant to  $\beta$ -lactams, aminoglycosides and quinolones including flouoroquinolones have arisen (Pagani *et al.* 2005).

*Klebsiella pneumoniae*, like other members of the enterobacteriaceae are important causative agents of hospital infections typically associated with pneumoniae, blood stream infections, urinary tract infections, bacteriamia and other intra – abdominal infections (Grover *et al.* 2006; Sun *et al.* 2006). According to Montgomerie (1979), *Klebsiella pneumoniae* infections can occur at nearly any body site, although urinary tract infections (UTI) and infections of the respiratory tract predominate.

This study was therefore carried out to investigate the prevalence of UTIs among students, as well as antimicrobial drug resistance in *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from urine samples of students of a university campus. These students were not on admission in hospitals, and have not been admitted in the hospital in the past 6 months prior to the period of this research work. The samples therefore were non-clinical urine samples.

## Materials and Methods:

### Research subjects and design

A total of 120 early morning urine samples collected from clinically healthy students were examined; 80 and 40 samples were collected from the female and male students respectively. Samples were processed within 1 hour of collection. All the samples were examined for the presence of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by standard procedures. Standard biochemical tests were employed for confirmation of the organisms. These included morphological characteristics for both

organisms, IMViC test procedures for *K. pneumoniae* and oxidase, urease, motility and sugar fermentation tests for *P. aeruginosa*.

### Antimicrobial resistance testing

Antimicrobial resistance testing was performed by the disk-diffusion tests on Mueller-Hinton agar, using antibiotic discs purchased from the market. Results were interpreted as susceptible or resistant according to the criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS), (2000). The following antimicrobials were tested: augmentin (30 $\mu$ g), amoxicillin (30 $\mu$ g), co-trimoxazole (25 $\mu$ g), chloramphenicol (30 $\mu$ g), ofloxacin (10 $\mu$ g), gentamycin (10 $\mu$ g), perfloxacin (10 $\mu$ g), streptomycin (30 $\mu$ g), sparfloxacin (10 $\mu$ g), ciprofloxacin (5 $\mu$ g), nalidixic acid (30 $\mu$ g), ampicillin (10 $\mu$ g) and cephalexin (30 $\mu$ g).

### Statistical analysis

The differences in resistance rates between genders were analysed using the *t*-test from excel. Results were considered significant at  $p < 0.05$ .

### Results

Out of the 120 urine samples, 96 (80%) were positive for *Pseudomonas aeruginosa*, comprising 62 from females and 34 from the male samples. On the other hand, 66 samples (55%) yielded *Klebsiella pneumoniae* isolates, with 48 and 18 coming from the female and male samples respectively.

An analysis of the antimicrobial resistance rates showed that the *P. aeruginosa* isolates were most resistant to ciprofloxacin (74%), while resistance to ofloxacin, gentamycin, perfloxacin, streptomycin, sparfloxacin and were moderate (19-43%). Resistance to augmentin, amoxicillin, co-trimoxazole and chloramphenicol were low (6-14%) as shown in Table 1.

For the *Klebsiella pneumoniae* isolates, resistance rates were moderate for chloramphenicol, amoxicillin, co-trimoxazole, and augmentin (18-28%), while of resistance were low for streptomycin, ciprofloxacin, nalidixic acid, Ampicillin, cephalexin, gentamycin, ofloxacin and sparfloxacin (5-12%) as shown in Table 2.

A comparative analysis of resistance rates showed that the *Pseudomonas* isolates exhibited greater resistance to more of the test antibiotics than the *Klebsiella* isolates. In about four of the test antibiotics however, which include, augmentin, amoxicillin, co-trimoxazole and chloramphenicol, *Klebsiella* isolates exhibited higher rates of resistance (Figure 1).

Result of statistical analysis by t-test however showed that there was no significant difference in resistance between the two groups of isolates.

The results of the multiple resistance analysis showed that the *Pseudomonas* isolates exhibited higher multiple resistance than the *Klebsiella* isolates for the same antibiotics. In all 75 (78.1%) of all *Pseudomonas* isolates were resistant to more than two antibiotics, the largest bloc being 29 isolates (30.2%) being which were resistant to 3 antibiotics. For *Klebsiella*, 50 isolates (75.8%) were resistant to more than 2 antibiotics. The largest bloc

here was 18 isolates (27.3%), which were resistant to 5 antibiotics. (Figure 2)

Analysis of prevalence shows that *Pseudomonas aeruginosa* was more prevalent among the students (80%) than *Klebsiella pneumoniae* (55%), (Figure 2). In addition, both isolates occurred more among the female students (64.6% and 72.7%) for *Pseudomonas* and *Klebsiella* isolates respectively compared to (35.4% and 27.2%) for the male students.

*Klebsiella* however occurred more with the female students than *Pseudomonas*, while the reverse was the case for the male students (Figures 3 and 4).

Table 1: Frequency of antimicrobial resistance rates in *Pseudomonas aeruginosa* isolates of non-clinical urine samples.

Antibiotics	No (%) of resistant isolates per sex		Total isolates N=96
	Females n=62	Males N=34	
Ciprofloxacin	59(95.2)	15(44.1)	74(77.1)
Sparfloxacin	30(48.4)	13(38.2)	43(44.8)
Streptomycin	27(43.5)	12(35.3)	39(40.6)
Perfloxacin	22(35.5)	13(38.2)	35(36.5)
Gentamycin	18(29.0)	10(29.4)	28(29.2)
Ofloxacin	11(17.7)	8(23.5)	19(19.8)
Chloramphenicol	8(12.9)	6(17.6)	14(14.6)
Co-trimoxazole	10(16.1)	4(11.8)	14(14.6)
Amoxicillin	8(12.9)	4(11.8)	12(12.5)
Augmentin	3(4.80)	3(8.80)	6(6.30)

Table 2: Frequency of antimicrobial resistance rates in *Klebsiella pneumoniae* isolates of non-clinical urine samples.

Antibiotics	No (%) of isolates per sex		Total isolates (n=66)
	Females (n=48)	Males (n=18)	
Co-trimoxazole	23(47.9)	6(33.3)	29(43.9)
Augmentin	23(47.9)	5(27.8)	28(42.4)
Amoxicillin	17(35.4)	5(27.8)	22(33.3)
Chloramphenicol	12(25)	6(33.3)	18(27.2)
Streptomycin	11(22.9)	1(12.5)	12(18.2)
Nalidixic acid	9(18.8)	3(16.7)	12(18.2)
Ciprofloxacin	10(20.8)	1(12.5)	11(16.7)
Gentamycin	9(18.8)	2(11.1)	11(16.7)
Ampicillin	7(14.6)	3(16.7)	10(15.2)
Cephalexin	5(10.4)	2(11.1)	7(10.6)
Sparfloxacin	5(10.4)	2(11.1)	7(10.6)
Ofloxacin	3(10.4)	2(11.1)	5(7.6)

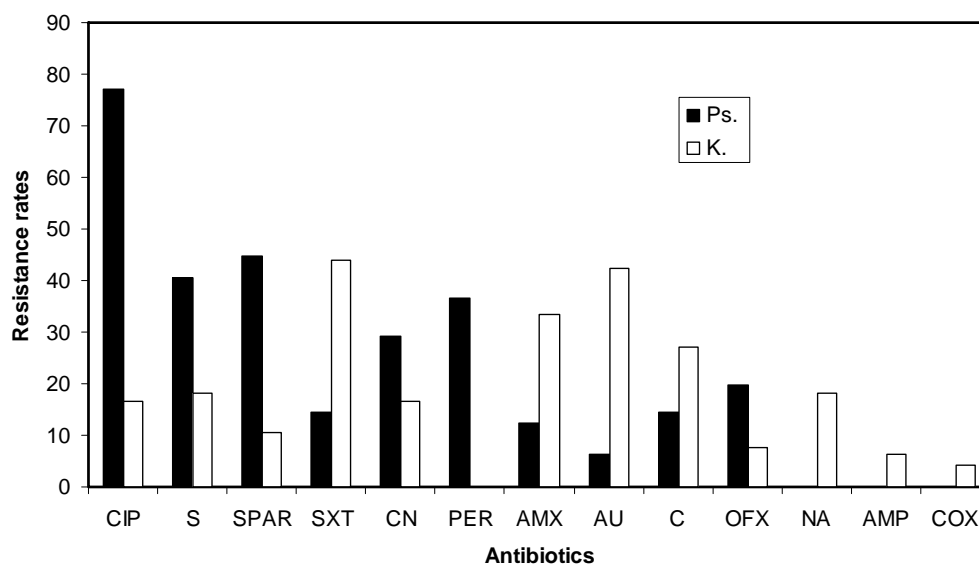


Figure 1: Comparative rates of resistance between the isolates for similar antibiotics

KEY: CIP, ciprofloxacin; S, streptomycin; SPAR, sparfloxacin; SXT, co-trimoxazole; CN, Gentamicin; PER, perfloxacin; AMX, amoxicilline; AU, augmentine; C, chloramphenicol; OFX, ofloxacin; NA, nalidixic acid; AMP, Ampicillin, COX, cephalixin. Ps, *Pseudomonas aeruginosa*; K, *Klebsiella pneumoniae*

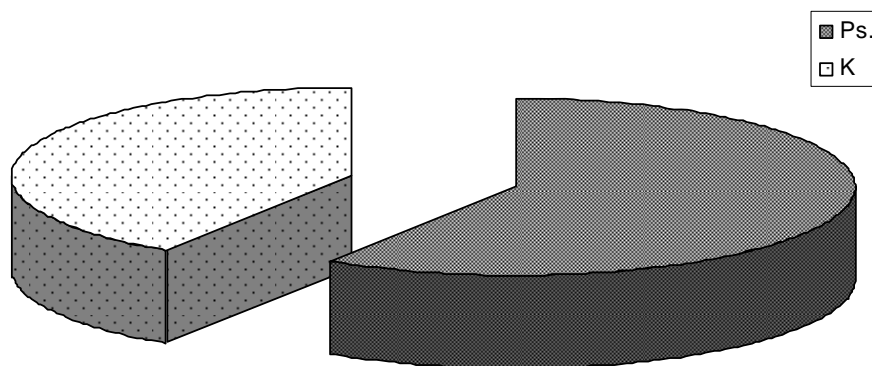


Figure 2: Total prevalence for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates from non-clinical urine samples

KEY: Ps, *Pseudomonas aeruginosa*; K, *Klebsiella pneumoniae*

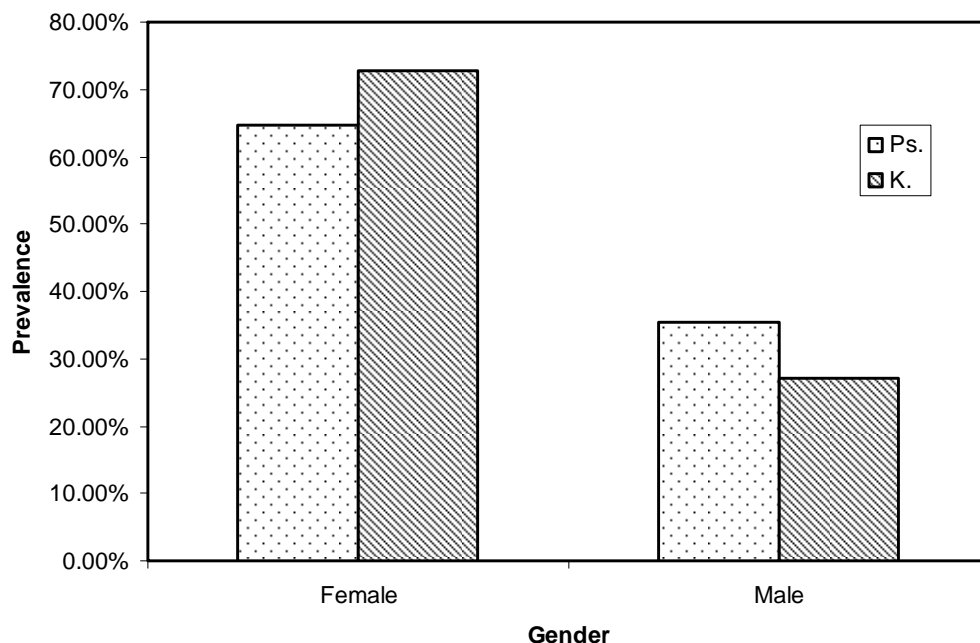


Figure 3: Prevalence for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates per gender.

KEY: Ps, *Pseudomonas aeruginosa*; K, *Klebsiella pneumoniae*

### Discussion

The *pseudomonas aeruginosa* isolates in this study were most resistant to ciprofloxacin. Even though the isolates were from apparently healthy individuals, they exhibited similar resistance trends as those isolated from hospital patients, as reported by Bouza *et al.* (1999), Cavallo *et al.* (2000) and Strateva *et al.* (2007). Bouza *et al.* (1999) reported further that resistance to ciprofloxacin by their isolates was more in out patients than in the nosocomial isolates. The incidence of resistance seems therefore to be dependent on the patterns of antibiotic usage.

Although *P. aeruginosa* strains exhibits an intrinsic sensitivity to  $\beta$ -lactams, imipenem, aminoglycosides and flouroquinolones, *P. aeruginosa* resistant to these antibiotics have emerged and is widespread (Pagani *et al.* 2005). Considering the relationship between antibiotic usage and enhanced development of resistance to antibiotics, the results obtained in this work attest to probable increased usage of antibiotics by the students in form of self medication. The students

were not on antibiotic prescription during the period of the study. The incidence of resistance seems therefore to be dependent on the patterns of antibiotic usage.

The isolates also showed only moderate resistance to the aminoglycosides tested in this study. This is in contrast to Anjun and Mir, (2010) and Strateva *et al.* (2007), where the resistance rates of their hospital isolates to gentamicin were much higher at 66% and 79.7% respectively. According to Boffi *et al.* (2000), substantial regional variation in resistance patterns has been observed and is probably related to antibiotic treatment regimen.

For the *Klebsiella pneumoniae* isolates, resistance to the majority of the antimicrobials fell between low and moderate. However, the quinolones including the flouroquinolones and aminoglycosides were most active against the isolates. This is in total agreement with Prado *et al.* (2007), whose isolates (both ES $\beta$ -L and non ES $\beta$ -L producers), even though environmental isolates, were most susceptible to the quinolones and aminoglycosides. Ramazanzadeh (2010) however reported ciprofloxacin resistance in

60% of his ES $\beta$ -L positive *Klebsiella* isolates and 13.6% in the ES $\beta$ -L negative isolates. Resistance to aminoglycosides, ciprofloxacin and co-trimoxazole in *K. pneumoniae* is high in ES $\beta$ -L positive isolates (Tonkic *et al.*, 2005). Our isolates therefore, could be potential ES $\beta$ -L producers, taking into account also, its resistance to the  $\beta$ -lactamase inhibitor, augmentin.

*Pseudomonas aeruginosa* was more prevalent (80%) in the urine samples examined than *Klebsiella pneumoniae* (55%). For both organisms however, prevalence was higher in females than in the male samples. This level of prevalence agrees with Anjun and Mir (2010), who reported a similar trend with regards to sex. This increased prevalence in females could be attributed to the differences in physiology between the males and females, where the urethra is short, and the distance between the anal and vaginal opening is small. Bacteria therefore easily invade and colonize the urinary tracts.

The high prevalence as well as high rates of resistance in the organisms isolated in this study, especially to ciprofloxacin, attests to some measure of antimicrobial abuse and poor hygiene among this group of individuals. Considering that the subjects were neither in- nor out- patients of hospitals, and yet had results comparing very closely with results of patients may mean that a large scale abuse of antimicrobials is going on. Abuse – overuse and misuse – is one of the greatest contributing factors to the selection and persistence of antimicrobial resistance among bacterial pathogens.

Results of a similar research conducted with female students residing in the campus of a university by Ojo and Anibijuwon (2010), also reported that 65% of the urine samples of the students tested had significant bacterial growth. They also noted the uncontrolled consumption of antibiotics among the students.

### Conclusion

The students had a high prevalence of UTIs amongst them, considering that at least 55% of the samples had bacterial growth. The *P. aeruginosa* isolates also exhibited higher resistance rates than the *K. pneumoniae* isolates. This is probably because *P. aeruginosa* has more tolerance to a wider variety of environmental conditions as well as resistance to a greater number of antibiotics. Untreated UTIs may also lead to septicaemia.

It is important therefore for enlightenment campaigns to be carried out among students and young adult populations on the dangers of moving about with untreated urinary tract infections as well as self medication with antimicrobials.

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