

# Prevalence and Antibiotic Resistance Pattern of *Escherichia coli* and *Klebsiella pneumoniae* in Urine Tract Infections at the La Paz Medical Center, Malabo, Equatorial Guinea

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## Abstract

The study was conducted to isolate and determine the antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* from urine samples over a 2-year period (August 2013-September 2015) at the La Paz Medical Center, Malabo. A retrospective analysis of 785 urine culture samples over a 2-year period August 2013-September 2015 was carried out according to the routine protocol of urinalysis. Bacterial etiological agents were isolated from 155 (19.7%) samples with highest prevalence of *Escherichia coli* (55.5%) followed by *Klebsiella pneumoniae* (23.2%), *Proteus mirabilis* (4.5%), *Pseudomonas species* (3.2%), *Enterobacter species* (2.6%), *Enterococcus faecalis* (2.6%) and others species (8.4%). The *E. coli* and *K. pneumoniae* represent 78.7% of all isolated bacterial strains. The *E. coli* and *K. pneumoniae* isolates possess highly resistant to ampicillin, Trimethoprim/Sulfamethoxazole, Doxycycline, Amoxicicline/Clavulanic acid. Whereas *K. pneumoniae* demonstrated also to be highly resistant to Gentamycin, Cefuroxime and Ceftriaxon, low level of resistance to Piperacilin/Tazobactam, Amikacin and the lowest to Imipenem. The alarming level of MDR strains to the first choice antibiotics treatment was observed.

## Keywords

Urine Tract Infections, Antibiotic Resistance, *Klebsiella pneumoniae*, *Escherichia coli*

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## 1. Introduction

Complicated Urinary tract infections (UTIs) are serious health affecting problems worldwide [1]-[3]. *E. coli*, *K.*

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*pneumonia*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *S. saprophyticus* are most common bacteria causing UTIs in human beings [4] [5]. Among the uropathogenic bacteria, *Escherichia coli* is predominant in both community and nosocomial UTI [2] [3] [6]-[15]. Infections are gradually becoming more and more difficult to treat and may lead to therapeutic dead ends. These resistance patterns have shown large inter-regional variability. Understanding the spectrum and resistance patterns may help guide effective empirical antibiotic therapies, decrease treatment failure and costs. Resistance patterns of microorganisms vary from country to country, state to state, large hospital to small hospital and hospital to community [1] [6]-[14]. In Equatorial Guinea, the problem of antibiotic resistance is compounding because of overuse and misuse of antibiotics. It is a first study of antibiotic resistance of uropathogenic bacteria in Equatorial Guinea.

The aim of this study was to assess the diversity and state of resistance of dominant uropathogenic bacteria such as *E. coli* and *K. pneumoniae* to antibiotics in Equatorial Guinea.

## 2. Subjects and Methods

The observational and prospective study was conducted at La Paz Medical Center, Malabo, Equatorial Guinea. We cultured and analysed 785 urine samples from patients of different departments at La Paz Medical Center. These samples were quickly recorded and processed according to the routine protocol of urinalysis.

### 2.1. Samples Processing

Urine sample (0.01 mL) was inoculated on Tryptic Soy Agar with 5% Blood sheep (TSAB), MacConkey's agar and CHROME Orientation agar (HyLabs Ltd.) by spread plate technique. The isolated bacteria were then identified by using Gram Stain and their biochemical characteristics using Remelrap ID system kits.

### 2.2. Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined using the disc diffusion method on Mueller Hinton agar according to the Guidelines of the Clinical Laboratory Standards Institute (GCLSI) [16]-[18].

Different families of antibiotics (discs obtained from OXOID) were studied such as: Cephems (cefuroxime, ceftriaxone); Penicillins (Ampicillin); Beta-lactam + inhibitor (Amoxicillin/Clavulanic acid, Piperacilin/Tazobactam); fluo quinolones (Ciprofloxacin); Aminoglycosides (Gentamicin, Amikacin); Tetracyclins (Doxycyclin); Trimethoprim/Sulfamethoxazole; Carbapenemes (Imipenem)

After incubation, the diameter of the inhibition zone formed around the disc was measured and compared to the critical values "d" and "D" of each antibiotic disc (according to CLSI) to qualify the target bacteria as sensitive (diameter of the inhibition > D) or resistant (diameter of the inhibition < d) or again intermediate (d < diameter of the inhibition < D). Multi Resistant strains were divided into MDR (Multiple Drug-Resistant), XDR (Extensively Drug-Resistant) and PDR (Pandrug-resistant) according to the Center for Disease prevention and Control [19]. MDR bacteria are defined as resistant to at least three different classes of antibiotics. XDR bacteria are characterized by their sensitivity to only one class of antibiotics and the PDR bacteria are resistant to all classes of antibiotics recommended for treatment [20].

## 3. Results

### 3.1. Epidemiology of Urinary Tract Infections

The total of 785 urine samples of both outpatients and in-patients of La Paz Medical Center was determined. 443 (55%) were from males while 342 (45%) were from females. A total of 155 bacteria strains (19.7%) were successfully isolated from urine samples. 76 (17.3%) positive sample were belong to males and 79 (23.1%) were belong to females. Out of 155 positive samples the *E. coli* (56%) isolation was the highest followed by *Klebsiella pneumonia* (24%), *Proteus mirabilis* (5.3%), and *Pseudomonas aeruginosa* (2.7%) and others (Table 1). Both *E. coli* and *Klebsiella pneumonia* isolates represent 78.7% of all isolated bacterial strains. Therefore, antibiotic resistance rates were performed specifically these two strains.

### 3.2. Antibiotic Resistance

The comparison of antibiotic resistance rates was performed specifically for *E. coli* and *K. pneumonia* (Table 2

**Table 1.** Profile prevalence of uropathogenic bacteria isolated.

Bacteria isolated	Total number	(%)
<i>Escherichia coli</i>	86	55.5
<i>Klebsiella pneumonia</i>	36	23.2
<i>Enterobacter</i> sp.	4	2.6
<i>Proteus mirabilis</i>	7	4.5
<i>Pseudomonas aeruginosa</i>	5	3.2
<i>Serratia</i> sp.	2	1.3
<i>Enterococcus faecalis</i>	4	2.6
<i>Staphylococcus aureus</i>	2	1.3
<i>Klebsiella oxytoca</i>	1	0.6
<i>Staphylococcus saprophyticus</i>	2	1.3
<i>Streptococcus agalactiae</i>	4	2.6
<i>Morganella morganii</i>	1	0.6
<i>Providencia</i> sp.	1	0.6

**Table 2.** Antibiotic resistance rates for *E. coli* isolates.

Antibiotic subclass	Antibiotic	<i>Resistant</i>		<i>Intermediate</i>		<i>Sensitive</i>	
		n	%	n	%	n	%
Penicillins	Ampicillin	50	100	0	0	0	0
	Amoxicillin/clavulanic acid	57	74.1	11	14.3	9	11.7
Beta-lactam + inhibitor	Piperacilin/tazobactam	7	8.4	11	13.3	65	78.3
	Cephalosporin II	44	64.2	9	13.4	16	22.4
Cephalosporin III	Ceftriaxone	51	66.2	0	0	27	33.8
Fluoroquinolones	Ciprofloxacin	44	53.7	5	6.1	33	40.2
	Aminoglycosides	Gentamicin	27	50.9	1.9	2.2	25
Tetracyclines	Amikacin	2	4.9	5	12.2	34	82.9
	Doxycycline	29	78.4	1	2.7	7	18.9
Folate pathway inhibitors	Trimethoprim/sulfamethoxazole	19	95	0	0	1	5.0
Carbopenemes	Imipinem	0	0	0	0	39	100

and **Table 3**). Very high rates of resistance to Ampicillin, Trimethoprim/Sulfamethoxazole, Doxycycline, Amoxicillin/Clavulanic acid were observed. *E. coli* exhibited the highest resistance to Ampicillin (100%), Trimethoprim/Sulfamethoxazole (95%), followed by intermediate level of resistance to Doxycycline (78.4%), Amoxicillin/Clavulanic acid (74.1%), Ceftriaxone (66.2%), Cefuroxime(64.2%), Ciprofloxacin (53.7%), Gentamicin (50.9%) and low level of resistance to Piperacilin/Tazobactam (8.4%), Amikacin (4.9%). It was not found *E. coli* isolates resistant to Imipinem. (**Table 2**) Whereas *K. pneumonia* presented the highest resistance to Ampicillin (100%), Trimethoprim/Sulfamethoxazole (100%), Amoxicillin/Clavulanic acid (93.3%) followed by highly resistance to Gentamycin (86.2%), Doxycycline (85.8%), Cefuroxime (85.7%), Ceftriaxone/Cefotaxime (81.3%), followed by intermediate level of resistance to Ciprofloxacin (62.5%), and low level of resistance to Amikacin (25.9%), Piperacilin/Tazobactam (12.5%) and the lowest to Imipinem (3.3%) (**Table 3**).

### 3.3. Multi-Resistant Strains

Based on their phenotypes, multi-resistant strains were classified as MDR, possible Extensively Drug-Resistant (XDR) or possible Pandrug-resistant (PDR) [19] [20]. The 74.4% of *E. coli* 91.7% of *K. pneumonia* were MDR strains. Whereas *K. pneumonia* presented a Extensively Drug-Resistant in 33.3% of the cases compared with only a 7% of *E. coli*. And 1 isolate of *K. pneumoniae* presented the PDR to all antimicrobial agents (**Table 4**).

**Table 3.** Antibiotic resistance rates for *K. pneumoniae* isolates.

Antibiotic subclass	Antibiotic	<i>Resistant</i>		<i>Intermediate</i>		<i>Sensitive</i>	
		n	%	n	%	n	%
Beta-lactam + inhibitor	Penicillins Ampicillin	15	100	0	0	0	0
	Amoxicillin/clavulanic acid	28	93.3	1	3.3	1	3.3
	Piperacilin/tazobactam	4	12.5	8	25.0	20	62.5
Cephalosporin II	Cefuroxime	24	85.7	2	7.1	2	7.1
Cephalosporin III	Ceftriaxone	26	81.3	0	-	6	18.7
Fluoroquinolones	Ciprofloxacin	20	62.5	8	25.0	4	12.5
Aminoglycosides	Gentamicin	25	86.2	0	0	4	13.8
	Amikacin	7	25.9	0	0	20	74.1
Tetracyclines	Doxycycline	18	85.8	1	1.9	2	9.5
Folate pathway inhibitors	Trimethoprim/sulfamethoxazole	13	100	0	-	0	-
Carbopenemes	Imipinem	1	3.3	0	-	29	96.7

**Table 4.** Percentage of multidrug resistance for each identified species.

Isolates	Total number	MDR	(%)	XDR	(%)	PDR	(%)
<i>E. coli</i>	86	62	74.4	6	7.0	0	0
<i>K. pneumoniae</i>	36	33	91.7	12	33.3	1	2.8

## 4. Discussion

Urine tract infection caused by microorganisms is one of the most common infections in the world [2]-[4]. Increasing the high resistance to broad spectrum antibiotics of urine pathogens especially *E. coli* and *K. pneumoniae* as the prevalence UTIs pathogens is alarming [2] [5] [21]-[23]. The rapid and correct choice of the antibiotic enables rapid cure of the patient, and sometimes even to save the patient's life. So it is very important to determine the antibiotic resistance patterns of UTIs pathogens. According to the Antimicrobial Resistance Global Report of WHO the information and data about antibiotic resistance situation obtained from the most African countries are still not enough or clear [2]. Our study is the first one about an antibiotic resistance pattern of prevalence UTI bacteria in Equatorial Guinea. In this study we compared the diversity of uropathogens isolated at La Paz Medical Center (Malabo, Equatorial Guinea) from 2013 to 2015 to that reported in similar studies from other African countries. According to this study out of 155 (19.7%) cultures were positive. 76 (17.2%) and 79 (23.1%) were isolated from male and female respectively. The finding that positive urine samples were more frequent in women than men is in agreement with previous studies [7] [11] [14]. The rate of positive urine samples from this study was 19.7%, which is lower than figures from previous studies [5] [7] [10] [14] in Ivory Coast (25.1%), Ethiopia (27.4%), South Africa (51%), and Cameroon (58.3%). It is however, higher than in Nigeria (13.1%) and Ghana (9.5%) [11] [22]. The difference in rates of positive samples can be explained by the differences in location and health situation in the region, capacity of hospital and the status of the patient (patient has the acute UTI or after antibiotic treatment). Our study showed that *E. coli* and by *Klebsiella pneumoniae* were implicated in 78.7% of all Urine Tract Infection pathogens. As expected, the rate of *E. coli* (55.5%) isolation was the highest followed by *Klebsiella pneumoniae* (23.2%). This is consistent with other studies in different African countries (Table 5). The prevalence of *K. pneumoniae* (23.2%) is similar to those reported from studies in Maroco (22%) [6] Nigeria (25%) [11], Ghana (26%) [22], and more highly from studies in other countries of African continent (Table 5).

### 4.1. Antibiotic Resistance

The antimicrobial resistance profile of isolates in our study revealed a generally higher resistance rate than reported in African studies [1]. The almost 100% resistance of *E. coli* and *K. pneumoniae* to Ampicillin and Trimethoprim/Sulfamethoxazole is the highest rate in African continent, except Nigeria and Morocco where were

**Table 5.** The prevalence of *E. coli* and *K. pneumoniae* isolates in African countries.

Country	<i>E. coli</i> (%)	<i>K. pneumoniae</i> (%)
Guinea Equatorial	55.5	23.2
Maroco [1]	63.0	22.0
Ethiopia [2]	60.3	5.9
Central African Republic [3]	55.6	16.9
Madagascar [4]	40.3	11.2
Ivory Coast [5]	28.7	14.9
Nigeria [6]	37.0	25.0
Gabonb [7]	29.5	10.3
Zimbabwe [8]	40.3	11.2
Ghana [11]	36.8	26.3
South Africa [22]	39.0	20.8
Cameroon [9]	31.4	1.2

shown similar results. The many studies [1] [3] [8] [10] [23] show the efficiency of Amoxicillin/Clavulanic acid against *E. coli* and/or *K. pneumoniae* strains, but the our data showed a lower effectiveness of this antibiotic for treatment of *E. coli* and *K. pneumoniae* isolates with their rate of resistance 74.1% and 93.3% respectively. Moreover, our results have shown a high rate of resistance to antibiotics belonging to groups such as Second and Third generation of cephalosporins (Cefuroxime, Ceftriaxone), Tetracyclins (Doxycycline), Fluoroquinolones (Ciprofloxacin) and aminoglycoside (Gentamicin) as observed in Africa [1]-[3] [5]-[13] [23] and in Europe. Our data is partially agrees with only studies in Nigeria [11] and Maroco [6].

Our study showed inefficiency to the first choice line drugs for the treatment of urine tract infection due to *E. coli* and *K. pneumoniae* isolates and this information is alarming. Just Imipenem was broadly the most sensitive drug, followed by Piperacilin/Tazobactam and Amikacin. The explanation is probably the fact that these are very powerful drugs used only in hospital settings and not as first-line therapy.

#### 4.2. Multi Drug Resistance

The present study showed that the prevalence of MDR among *E. coli* (74.4%) and *K. pneumoniae* (91.7%) isolates was higher compared to the study carried out in Ivory Coast [5] where the prevalence of MDR *E. coli* and *K. pneumoniae* was 14.4% and 23.1% respectively. Our ratio of MDR *E. coli* isolates was relatively lower than those reported in other African countries such as Sudan (92.2%) [21], Ethiopia (87.4%) [23] and Nigeria (83.9%) [11]. Like other studies elsewhere, our MDR *E. coli* and *K. pneumoniae* isolates were found sensitive to Imipenem, Piperacilin/Tazobactam and Amikacin.

#### 5. Conclusion

Our study has shown an overview of the common uropathogens bacteria founded in Equatorial Guinea compared to others African countries. *Escherichia coli* and *Klebsiella pneumoniae* were the most predominant strains in urine tract infection (UTI). The high level of Multi Drug Resistant (MDR) strains and the emergence level of Extensively Drug-Resistant (XDR) strains to the first choice antibiotics were observed.

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#### Conflict of Interest

The author declares that he has no competing interests.

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