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Research Article

Antibiotic Susceptibility Profile of Extended-Spectrum Beta-Lactamase in Escherichia Coli and Klebsiella Pneumonia from Urine Samples at Rwanda Military Hospital

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Abstract

Background: Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* have emerged as significant pathogens in urinary tract infections (UTIs) due to their ability to resist commonly used antibiotics, leading to challenging treatment scenarios.

Aim: This study aimed to determine the ESBL-producing *E. coli* and *K. pneumonia* and antibiotic susceptibility patterns of ESBL producing *E. coli* and *K. pneumoniae* isolates obtained from patients with UTI and to evaluate the effectiveness of various antibiotics against these bacteria.

Methods: This study used retrospective method to determine the ESBL-producing *E. coli* and *K. pneumonia* and antibiotic susceptibility patterns of ESBL producing *E. coli* and *K. pneumoniae* isolates obtained from patients with UTI and to evaluate the effectiveness of various antibiotics against these bacteria.

Results: The findings from this study revealed a higher frequency of ESBL-producing *E. coli* and *K. pneumoniae* in males (58%) than in females (42%). Specifically, *E. coli* accounted for 82% of the ESBL-producing isolates, while *K. pneumoniae* represented 18%. This study found that antibiotics such as Cefotaxime, Ceftazidime, and Ceftriaxone were notably less effective against UTIs caused by these bacteria. The resistance rates to Cefotaxime, Ceftazidime, and Ceftriaxone were 92%, 93%, and 94% for *E. coli,* and 78%, 83%, and 92% for *K. pneumoniae*, respectively. This underscores the growing ineffectiveness of these beta-lactam antibiotics in treating infections caused by ESBL-producing strains. Conversely, this study observed that Meropenem and Piperacillin-Tazobactam exhibited relatively higher efficacy. Specifically, Meropenem was effective in 65% of *E. coli* and 62% of *K. pneumoniae* infections, while Piperacillin-Tazobactam was effective in 71% of *E. coli* and 50% of *K. pneumoniae* infections. These results are crucial for guiding empirical therapy and tailoring treatment regimens for UTIs caused by ESBL-producing bacteria. Given the high prevalence of resistance to third and fourth generation beta-lactam antibiotics, the findings from this study emphasize the need for ongoing surveillance of antibiotic susceptibility patterns and the prudent use of Meropenem and Piperacillin-Tazobactam to manage infections effectively. Additionally, higher prevalence in males could suggest gender-based differences in susceptibility or access to healthcare, warranting further investigation to understand the underlying factors contributing to these discrepancies.

Keywords: Antibiotic Susceptibility Profile; Extended Spectrum Beta Lactamase; *Escherichia Coli*; *Klebsiella Pneumonia.*

1. INTRODUCTION

Extended Spectrum Beta lactamases (ESBLs) are a group of plasmid-mediated β-lactamases rapidly evolving enzymes that are capable of hydrolyzing the third and fourth generation cephalosporins, monobactams, penicillin and sometimes carbapenems yet are inhibited by β-lactamase inhibitors. Extended Spectrum Beta lactamases (ESBLs) producing gram negative bacilli exhibit resistance against many classes of antibiotics, resulting in limitation of therapeutic options, posing a therapeutic challenge today in the treatment of hospitalized and community-based patients. Infections due to ESBL producing strains range from uncomplicated urinary tract infections to life-threatening sepsis[1]

To survive the effects of antibiotics, some bacteria are constantly finding new defense strategies, called "resistance mechanisms. This resistance leads to a condition where fewer antibiotic options are available to treat bacterial infections, especially those caused by beta lactamase-producing strains. In many cases, even common infections, such as urinary tract infections, caused by ESBL-producing bacteria require more complex treatments. On the other hand, instead of taking antibiotics orally at home, patients with these UTIs may

require hospitalization and intravenous (IV) carbapenem antibiotics[2]

Enterobacteriaceae, especially *Klebsiella* species producing ESBLs have been discovered since the 1980s as a major cause of hospital-acquired infections. However, during the late 1990s, several community-acquired microorganisms that commonly cause urinary tract infections and diarrhea have also been found to be ESBL producers and these include *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio cholerae*. ESBLs are often encoded by genes located on large plasmids, and these also carry genes for resistance to many antimicrobial agents such as aminoglycosides, tetracyclines, chloramphenicol and fluoroquinolone. Thus, very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing bacteria[3]

β-Lactams are a group of antibiotics acting on the cell wall of a bacterial cell inhibiting the growth of sensitive bacteria by inactivating enzymes located in the bacterial cell membrane, which are involved in cell wall synthesis by binding to essential penicillin-binding proteins (PBPs). These include penicillins, cephalosporins, carbapenems, and monobactams. These bind to and inhibit the carboxypeptidases and transpeptidases. Resistance to β-lactams may be inherent to a particular species, which has inherently insensitive PBPs, may be acquired through spontaneous mutation or DNA transfer. Functionally, β-lactam resistance may be a result of the production of β-lactamases, impermeability, efflux and target modification[4]

The Clinical and Laboratory Standards Institute (CLSI) recommends a phenotypic combined-disk test for ESBL production in Enterobacteriaceae. It consists of measuring the growth-inhibitory zones around both cefotaxime (CTX) and ceftazidime (CAZ) disks with or without clavulanate (CA) for *E. coli, K. pneumoniae*, and *Proteus mirabilis.* The phenotypic confirmatory tests for ESBL production include cephalosporin/clavulanate combination discs method demonstrating a synergistic activity between a cephalosporin and a beta-lactamase inhibitor. There is difficulty in identifying ESBL-producing organisms in many clinical laboratories, making it likely that their prevalence is underestimated and knowledge among clinicians still low[5]

Extended spectrum beta-lactamase (ESBL) producing *E. coli* and *K. pneumonia* are becoming increasingly prevalent causative agents of urinary tract infections (UTIs). Antibiotic susceptibility testing of these bacteria reveals a high level of resistance to important beta-lactam antibiotics which are commonly used for the treatment of UTIs. As a result, the identification and treatment of ESBL-producing bacteria remain a significant challenge in clinical settings globally[6]. This study focus aimed to assess antibiotic susceptibility profile of extended spectrum beta lactamase producing *E. coli* and *K. pneumonia* isolated from urine samples of both inpatients and outpatients attending Rwanda Military Hospital (RMH).

2. RESEARCH METHODOLOGY

2.1. Study area

This study was conducted in the bacteriology service of Pathology laboratory department of the Rwanda Military Hospital (RMH) located in Kigali city.

2.2. Study design and study period

This retrospective study was conducted among male and female patients who tested positive for ESBL-producing *E. coli* and *K. pneumoniae* and attended Rwanda Military Hospital for a urine antibiogram test. The study covered a four-month period from January to April 2024.

2.3. Study population and sample size

This study included 108 patients who met the inclusion criteria and attended Rwanda Military Hospital for urinalysis and antibiogram testing. All patients had positive test results for ESBL-producing *E. coli* and *K. pneumonia*. Only samples with complete information were included in this study.

2.4. Data collection

The tested results of bacteria and antimicrobial resistance of patients were collected from the medical records (laboratory register). All data with complete documentation were selected and included in this study. Accordingly, the sample size of 108 patients was obtained.

2.5. Data analysis and presentation

Data were analyzed using Microsoft Excel 2016 and Statistical Package for the Social Science (SPSS) version 22. Findings were presented in form of tables as frequency and/or percentages.

3. RESULTS AND DISCUSSION

3.1. Patient's **demographic characteristics**

Table 1 represents demographic characteristics of patients diagnosed with *Escherichia coli* and *Klebsiella pneumoniae* at Rwanda Military Hospital regarding age and gender.

Table 1*:* Demographic characteristics of study participants

Variables		Frequency (n)	Percent (%)
Gender	Female	45	41.7%
	Male	63	58.3%
Total		108	100.0%
Age	[<25]	18	16.7%
	[26-50]	42	38.9%
	[51-75]	37	34.3%
	[>76]	11	10.2%
Total		108	100.0%

Majority of diagnosed patients were male with a frequency of 63(58.3%), and the female patients was 45 (41.7%) of the total 108 (100%) of targeted patients. Among the age group from diagnosed patients mostly affected age range were between [26-50] years with 42 patients corresponding to (38.9%), followed by [51-75] years with 37 patients equivalent to (34.3%), then [< 25] years with 18 patients contributing (16.7%), the least affected age crust were noted among [> 75] years with 11 patients corresponds to (10.2%) of total 108(100%) diagnosed patients requested for bacteriological exams.

The analysis of incidence by age group revealed a low rate in pediatric patients [<25], with the highest incidence in adult patients aged between 51 and 75 years (34%) and [>76] (10%). These findings align with a study conducted by another researcher, who reported the highest infection rate in Italian individuals aged 65 years or older[7]. As noted in previous reports, patient gender is a risk factor in the distribution of ESBL producers[8]. In the present study, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was found more in males than females (58% and 42%). The results are comparable to the studies done in Iran[9].

3.2. Pathogen distribution

The table illustrates the distribution of *Escherichia coli* and *Klebsiella pneumoniae* across different gender and age groups. In analyzing the distribution of those bacterial pathogens, my study reveals that *E. coli* is significantly more prevalent than *K. pneumoniae* across both genders.

Escherichia coli accounts for 82.4% of cases, with a nearly equal distribution observed in both females (82.2%) and males (82.5%). This prevalence aligns with findings from recent studies highlighting *E. coli* as the predominant pathogen in various infections[10]. In contrast, *K. pneumoniae* constitutes a smaller portion of infections at 17.6%, with a slightly lower distribution in females (17.8%) compared to males (17.5%). This trend is consistent with current literature suggesting that *K. pneumoniae*, while significant, is less frequently encountered than *E. coli* in similar settings[10]. The marked predominance of *E. coli* over *K. pneumoniae* across genders underscores its role as a major pathogen in the population studied.

Table 2: Distribution of *E. coli* and *K. pneumonia* causing urinary tract infections according to age and gender

Variables	*Escherichia coli*	*Klebsiella pneumoniae*	Total
Gender			
Female	37(82.2)	8(17.8)	45(100)
Male	52(82.5)	11(17.5)	63(100)
Total	89(82.4)	19(17.6)	108(100)
Age			
[<25]	17(94.4)	1(5.6)	18(100)
[26-50]	32(76.2)	10(23.8)	42(100)
[51-75]	31(83.8)	6(16.2)	37(100)
[>76]	9(81.8)	2(18.2)	11(100)
Total	89(82.4)	19(17.6)	108(100)

Age-specific analysis reveals that *E. coli* is most common in individuals under 25 years (94.4%), it is found that *E. coli* infections are particularly prevalent among younger individuals, similar to my observation that the [<25] age group has the highest prevalence of *E. coli* and highlights the higher susceptibility of younger populations to *E. coli* infections, potentially due to different risk factors and exposure rates[11]. While *K. pneumoniae* is least frequent in this age group and peaks in the [26-50] years (23.8%) and these findings align with recent study which reported that *K. pneumoniae* infections are more common in middle-aged adults, and suggested that the increased prevalence in this age group could be attributed to chronic health conditions and changes in immune function[12].

3.3. Antibiotic Susceptibility pattern of isolated *E.coli* and *K. pneumonia* against antibiotics in diffusion disk test

This study showed very high levels of resistance to antibiotics among ESBL-producing gram-negative bacteria causing complicated UTIs. Carbapenems (meropenem) and piperacillin-tazobactam were the relatively effective agents against ESBL-positive *E. coli* and *K. pneumoniae* strains. This study is in the same line with the study conducted in Iran evaluation of antibiotic susceptibility pattern of extended spectrum beta lactamases producing bacteria. Furthermore, these bacteria are resistant not only to extended-spectrum cephalosporins including ceftazidime, ceftriaxone, cefepime, but also penicillins[13].

3.4. Antibiotic susceptibility pattern of *E. coli*

DIAGNOSIS	Antibiotic	n	Susceptibility, n(%)	
			Resistant	Sensitive
Escherichia coli	Cefotaxime	59	54(91.5)	5(8.5)
	Ceftazidime	69	65(94.2)	4(5.8)
	Ceftriaxone	52	43(82.7)	9(17.3)
	Meropenem	62	18(29)	44(71)
	Piperacillin tazobactam	71	25(35.2)	46(64.8)
	Cefepime	19	13(68.4)	6(31.6)
	Amoxicillin + clavulanic acid	63	47(74.6)	16(25.4)

Among the *E. coli* positive patients, there were 89 (82.4%) cases. The bacteria showed high resistance to the antibiotics ceftazidime (CAZ) and cefotaxime (CTX), with resistance rates of 65 (94%) and 54 (92%) patients, respectively, moderate resistance was observed on Amoxicillin+clavulanic acid (AMC) and Cefepime (CFM) resisted on 47(75%) and 13(68.4%) patients. The first sensitive antibiotic was Meropenem (MEM), which was effective in 44 (71%) patients, followed by Piperacillin/tazobactam (TZP), which was effective in 46 (65%) patients.

E. coli showed moderate sensitivity to two antibiotics among the antibiotics that were routinely used with notable percentages, Meropenem, and Piperacillin-tazobactam, with sensitivities of 71 % and 65% respectively. These results were similar to those obtained in the current study where Meropenem and piperacillin-tazobactam, produced bacteriologic improvement in <15% resistance. These results underscore their potential as effective treatment options for *E. coli* infections[14].

In contrast, CAZ and CTX face considerable challenges, with resistance rates of 83% and 92% respectively, underscoring the limited utility of these antibiotics in treating *E. coli* infections. This might be due to the extensive use and misuse of those drugs in the population. These results accentuate the urgent need for cautious antibiotic prescribing practices and the exploration of alternative treatment strategies to combat rising antibiotic resistance in *E. coli*. This study is similar to the study where the antimicrobial sensitivity analysis revealed low sensitivities towards ceftazidime, and cefotaxime (85.4 and 100%) respectively, suggesting that these drugs are no longer effective for ESBLs empirical[15].

3.5. Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

DIAGNOSIS	Antibiotic	n	Susceptibility, n(%)	
			Resistant	Sensitive
Klebsiella pneumoniae	Cefotaxime	14	13(92.9)	1(7.1)
	Ceftazidime	9	7(77.8)	2(22.2)
	Ceftriaxone	12	11(91.7)	1(8.3)
	Meropenem	10	5(50)	5(50)
	Piperacillin tazobactam	13	5(38.5)	8(61.5)
	Cefepime	7	6(85.7)	1(14.3)
	Amoxicillin + clavulanic acid	17	14(82.4)	3(17.6)

Among resistance on *K. pneumoniae* higher resistance was observed on the following antibiotics cefotaxime (CTX), ceftriaxone (CTR), and Amoxicillin+clavulanic acid (AMC) with 93%, 92%, and 83%, patients successively, moderate resistance was noted on ceftazidime (CAZ) and Cefepime (CFM) with 78% and 86% patients respectively. For sensitivity action, the mostly sensitive antibiotics were Piperacillin tazobactam (TZP) which was sensitive to 62% patients, and Meropenem (MEM) was next with 50% patients, of a total of 17.59% patients who were *K. pneumoniae* positive.

Klebsiella pneumonia also showed a high resistance pattern towards third and fourth-generation cephalosporins such as ceftriaxone, cefotaxime, cefepime, and Amoxicillin+clavulanic acid with corresponding percentages 92%, 93%, 86% and 83% which is similar to the one done by Siriphap, where the resistance rate of *K. pneumonia* and 71.3% for cefotaxime and ceftazidime, (100%) and ceftazidime (85.4%)[6]. Conversely, piperacillin-tazobactam exhibited moderate susceptibility at 62% and 50%, which is lower compared to the susceptibilities of 80% and 78%[16].

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3.6. Comparison of antibiotic susceptibility patterns of *E. coli* and *Klebsiella pneumoniae*

Figure 1 below presents a comparison of the antibiotic resistance and sensitivity profiles of *Escherichia coli* and *Klebsiella pneumoniae* based on the provided data, notable differences in resistance and sensitivity emerge, highlighting that *E. coli* generally exhibits higher resistance and sensitivity rates compared to *K. pneumoniae*, indicating a more challenging scenario for treatment with certain antibiotics

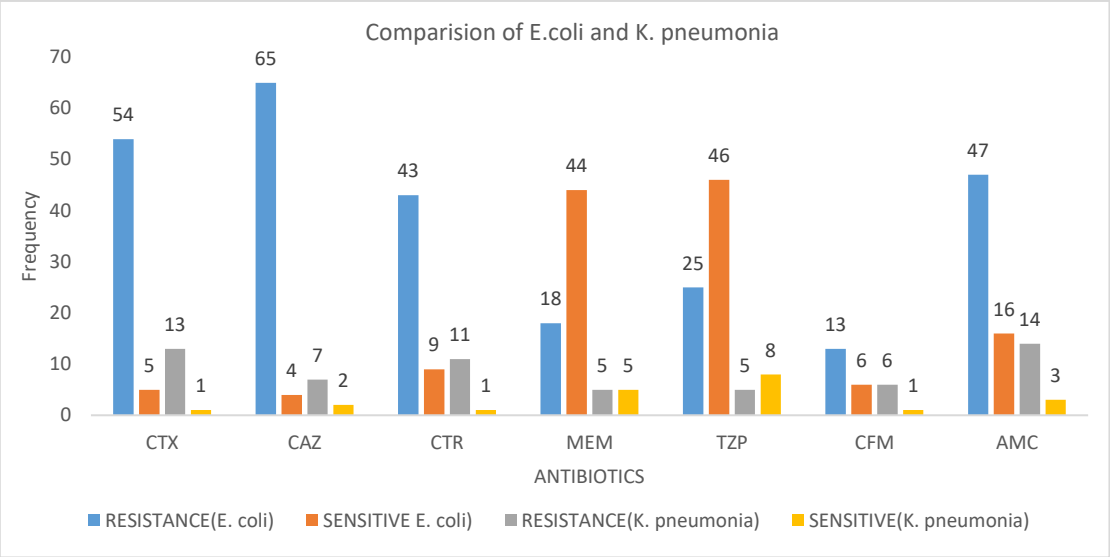

Figure 1: Antibiogram of both *E. coli* and *K. pneumonia*

The chart above demonstrates antibiotic susceptibility patterns of *E. coli* and *K. pneumonia*, the most resistance pattern was observed on *E. coli* with 69 patients on CAZ antibiotics followed by CTX with 54 patients, while the highest sensitivity pattern was noted in *E. coli* again with 44 patients on MEM and 46 on TZP, simply *E. coli* were having extended patterns of both resistance and sensitive than *K. pneumonia*.

Specifically, *E. coli* exhibits the highest resistance to ceftazidime, with 94.2% of isolates being resistant. This is in stark contrast to *K. pneumoniae*, where 77.8% of isolates are resistant to ceftazidime. A closer look at the data reveals that the highest resistance rate for *E. coli* is observed with ceftazidime (94.2%), and for *K. pneumoniae*, the highest resistance is to cefotaxime (92.9%). This significant difference in resistance patterns indicates that *E. coli* is more resistant to ceftazidime than *K. pneumoniae* is to cefotaxime. This finding aligns with recent studies that highlight increasing resistance trends in *E. coli*, particularly to cephalosporins like ceftazidime, which are crucial for treating various infections[11].

Moreover, *E. coli* also shows higher resistance to cefepime (68.4%) compared to *K. pneumoniae* (85.7%). While both pathogens exhibit significant resistance to cefepime, *E. coli*'s resistance rate is notably lower. This indicates that *K. pneumoniae* might pose a greater challenge in cases where cefepime is used as a treatment option. Research supports this observation, noting that cefepime resistance in *K. pneumoniae* has been increasing due to the spread of ESBLs[17].

On the sensitivity front, *E. coli* demonstrates higher sensitivity to Meropenem compared to *K. pneumoniae*. In *E. coli*, 71% of isolates are sensitive to Meropenem, while only 50% of *K. pneumoniae* isolates show sensitivity to this antibiotic. This suggests that Meropenem may be a more reliable treatment option for *E. coli* infections than for *K. pneumoniae*. Recent studies corroborate this, showing that meropenem remains one of the most effective treatments for resistant strains of *E. coli*[18].

Additionally, *E. coli* exhibits higher sensitivity to piperacillin-tazobactam (64.8%) compared to *K. pneumoniae* (61.5%). Although the difference is not as pronounced, it still indicates a trend where *E. coli* may respond better to piperacillin-tazobactam than *K. pneumoniae*. This finding is supported by contemporary research, which shows that piperacillin-tazobactam retains significant efficacy against *E. coli*, though resistance is emerging[19].

4. Conclusion

In conclusion, the findings underscore the significant prevalence of Extended Spectrum Beta Lactamase-producing organisms, primarily *E. coli* and *K. pneumoniae*, among which male patients are disproportionately affected. The high resistance rates observed against key antibiotics, including Amoxicillin/clavulanic acid, Cefotaxime, Ceftriaxone, and cefepime, highlight the urgent need for tailored antimicrobial strategies to combat these pathogens effectively.

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Hospital with a letter requesting data collection. Approval letter for data collection was obtained from Rwanda Military Hospital before data collection. All research methods were performed in accordance with the relevant guidelines and regulations. The data obtained was kept confidential and used for academic purposes.

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