Prevalence of Extended Spectrum Beta-Lactamase Producing *Klebsiella Pneumoniae* and *Escherichia Coli* Among Clinical Isolates in Lautech Teaching Hospital, Ogbomoso, Oyo State, Nigeria

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ABSTRACT

Klebsiella pneumoniae and *Escherichia coli* are major extended-spectrum beta-lactamase producing organism. Extended spectrum beta-lactamases (ESBLs) inactivate newer cephalosporins through hydrolysis, increasing therapeutic failure and antibiotic resistance worldwide. This study aimed to determine the prevalence of ESBL-production in clinical *Klebsiella pneumoniae* and *Escherichia coli* isolates. A total of one hundred and four (104) *Klebsiella pneumoniae* and *Escherichia coli* isolates from various clinical samples were obtained from Medical Microbiology Laboratories of LAUTECH Teaching Hospital. Isolates collected were recovered on MacConkey agar at 35°C and then identified using standard biochemical tests. They were further screened for antimicrobial susceptibility and resistance by disc diffusion method. Isolates that were resistant to at least two of the third-group cephalosporins such as ceftriaxone, cefotaxime and ceftazidime were confirmed as ESBL producers using Double Disks Synergy Test (DDST). Out of the one hundred and four (104) isolates screened, twenty (20) were confirmed by DDST to be ESBL producers. The prevalence rate of ESBL production in clinical *Klebsiella pneumoniae* and *Escherichia coli* isolates were observed to be 19.2%. The study established the prevalence of Extended Spectrum Beta-Lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* among clinical isolates in the study area. Therefore, monitoring dissemination and transmissions of ESBL producers are highly recommended for optimum patient care and preventing the spread of multidrug resistant (MDR) pathogens.

Keywords: Extended Spectrum Beta-Lactamase, Double Disks Synergy Test, Antimicrobial Susceptibility.

INTRODUCTION

The rise in antimicrobial resistance has evolved to become a pressing global health concern, with the production of enzymes such as Extended-spectrum Beta-lactamases (ESBL) contributing significantly to this crisis. ESBLs are a group of plasmids mediated, diverse and rapidly evolving enzymes that enable bacteria possessing them to hydrolyze and thus confer resistance to penicillin, extended-spectrum Cephalosporins and monobactams (Gundran *et al.,* 2020). In addition, the plasmid-encoded extended-spectrum beta-lactamase are easily transmitted from one bacteria to another by horizontal gene transfer (Nwafia *et al.,* 2019). As a result, most ESBL isolates are resistant to antimicrobials other than beta-lactams, such as aminoglycosides, fluroquinolones, tetracyclines and nitrofurantoin and trimethoprim/sulphamethoxazole (Maduakor *et al.,* 2022).

Among other Enterobacteriaceae, ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* have emerged as one of the greatest threats to human health, frequently implicated in hospital-acquired infections and community-onset infections (Iroha *et al.,* 2017). These organisms can hydrolyze a wide range of beta-lactams antibiotics, including third-generation Cephalosporins leading to limited therapeutic options and increased morbidity and mortality rates.

In resource limited countries, particularly countries in sub-Saharan Africa, the spread of these resistant bacteria among hospitalized patients would otherwise have devastating and debilitating effect considering the health infrastructure, lack of monitoring for microbial drug resistance, very low infection control practices, poor hygienic practices and the continued misuse and abuse of antibiotics (Essack *et al.,* 2017). To exacerbate the

situation, most clinical laboratories in these countries do not routinely screen for ESBL-producers, thus resulting in avoidable treatment failures in patients and outbreaks of resistant bacteria infections that require expensive control effects (Mofolorunsho *et al.,* 2021).

In Nigeria, like other developing countries, the magnitude of the problem associated with ESBL is still underestimated and not detected routinely in most clinical laboratories in the country (Olivia & Bernard, 2024). Although there are a few publications on detection of ESBL-producing bacteria causing clinical infections in many parts of the country, there's paucity of information regarding ESBLs producing organisms in Ogbomoso, Nigeria. Therefore, this study aimed to report the prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in LAUTECH Teaching Hospital Ogbomoso, Oyo state, Nigeria.

METHODOLOGY

Ethical consideration

Ethical approval was obtained from the Ethical Research Committee, Faculty of Basic Medical Sciences, Ogbomoso, Oyo state with Approval number ERCFBMSLAUTECH:099/03/2025

Study design

This cross-sectional hospital-based study was carried out at LAUTECH Teaching Hospital (LTH) Ogbomoso, Oyo state Nigeria. A total of One hundred and four (104) of clinical isolates of *E. coli* (n=53) and *K. pneumoniae* (n= 51) were obtained from the Department of Medical Microbiology and Parasitology at LTH Ogbomoso from different clinical specimens including urine, blood, stool, wound swab, vagina swab, sputum and semen between October and December 2024.

Inclusion Criteria

Identified clinical and non-duplicate pure culture isolates of *Klebsiella pneumoniae* and *Escherichia coli* from various samples, irrespective of the gender, were used in this work.

Exclusion Criteria

Identified clinical isolates other than *K. pneumoniae* and *E. coli* and duplicate cultures were excluded from this study.

Identification of Bacteria Isolates

The isolates were first sub-cultured into nutrients broth and subsequently recovered on MacConkey agar plates after they were incubated aerobically for 24hours at 37°C. They were identified based on their gram reactions and specific biochemical tests such as citrate and indole test. *Escherichia coli* are lactose fermenter, gram negative rods, motile, indole positive and citrate negative. *Klebsiella pneumoniae* are lactose fermenter, gram-negative bacilli, mucoid, non-motile, indole negative and citrate positive.

Phenotypic Detection of ESBL Production

Screening of Presumptive ESBL producing Isolates.

This encompasses screening for reduced susceptibility to more than one of the indicator antimicrobials (ceftazidime 30ug, ceftriaxone 30ug and cefotaxime 30ug). Using disc diffusion technique, a loopful of test isolates was suspended into a normal saline to match 0.5 McFarland turbidity standard and were swabbed onto the surface of dried Muller Hinton agar plates using sterile swab sticks. The mentioned antimicrobial discs were placed 20mm apart on a surface of the Muller Hinton agar using sterile forceps, leaving 15mm away from the edge of the petri dish. Following incubation at 37°C for 24hours, inhibition zones were measured to the nearest mm. Using Clinical and Laboratory Standard Institutes (CLSI), when a diameter zone of < 22mm for ceftazidime, <25mm for ceftriaxone and <27mm for cefotaxime were recorded, the isolates were reported as suspected ESBL and further subjected to ESBL confirmation

ESBL confirmatory method

Confirmatory test for ESBL production was done using the double disc synergy method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antibiotic discs of amoxicillin/clavulanic acid(20/10 ug) were put in between ceftazidime (30ug) and cefotaxime (30ug) at 20mm apart and incubated. Isolates that showed a clear zone of extension of either of ceftazidime or cefotaxime inhibition zone towards the amoxicillin/clavulanic acid(,20/10 ug) disc were considered ESBL producers.

RESULTS

Of the 104 clinical isolates that were obtained from the hospital laboratory, 51(49%) were identified to be *Klebsiella pneumoniae* and 53(51%) were identified to be *Escherichia coli*.

Table 1 and 2 presents the antibiotic susceptibility pattern of *K. pneumoniae* and *E.coli* isolates respectively. Results showed that the highest resistance (83%) to Ceftazidime (30ug) and (75.5%) to Ceftriaxone(30ug) were observed in *E.coli* and *K. pneumoniae* respectively, while the least resistance (30.6) to Cefotaxime(30ug) was recorded in *K. pneumoniae*. The highest susceptibility to Ceftazidime of 69.4% followed by Ceftriaxone (30ug) of 66% were observed in *K. pneumoniae* and *E. coli* respectively, while the least susceptibility to Ceftazidime (30ug) of 17% was observed in *E.coli*.

Table 1. Antibiotic susceptibility screening for *Klebsiella pneumoniae* isolates

Antibiotic	Sensitive (%)	Resistant (%)	Total	Chi-square value	P-value
CEFOTAXIME(CTX)	32 (63.3)	18 (36.7)	50	3.45	0.06
CEFTRIAXONE(CRO)	12 (24.5)	38 (75.5)	50	12.76	0.00
CEFTAZIDIME(CAZ)	35 (69.4)	15 (30.6)	50	7.37	0.01

Table 2. Antibiotic susceptibility screening for *Escherichia coli* isolates

Antibiotic	**Sensitive (S)**	**Resistant (R)**	**Total**	**Chi-square value**	**P-value**
CEFOTAXIME(CTX)	31(58.9)	22(41.1)	53	1.93	0.17
CEFTRIAXONE(CRO)	35(66)	18(34)	53	4.93	0.03
CEFTAZIDIME(CAZ)	9(17)	44(83)	53	22.24	0.00

ESBL producing Isolates

Expression of ESBL production was phenotypically detected using double disc synergy test. From the 104 isolates screened, the total number of ESBL-producing isolates was 20. Out of these ESBL producing isolates, *E.coli* accounted for 55% (11/20) whereas *K. pneumoniae* accounted for 45% (9/20). Hence, resulting to an overall prevalence of 19.2%(Table 3).

Table 3. Prevalence of ESBL producers

Isolates	No screened	No of ESBL confirmed	% of confirmed ESBL
E. coli	53	11	20.8
K. pneumoniae	51	9	17.6
Total	104	20	19.2%

DISCUSSION

The growing emergence and rapid spread of multi-drug-resistant pathogens represent significant public health threat, particularly in health-care settings; among them, Extended-Spectrum Beta-lactamases producing-*Enterobacteriaceae* has been a major concern. In recent years, following the extensive use of broad-spectrum cephalosporins, ESBL-producing Gram negative bacteria, especially *K. pneumoniae* and *E.coli* has emerged as serious pathogens in hospital and community acquired infections worldwide. Resource-limiting countries such as Nigeria are far behind in the fight against antimicrobial resistance as there are poor access to quality microbiology diagnostics, and lack of enforcement of infection control measures resulting in increased risk of morbidity and mortality from these multi-drug-resistant pathogens.

In this study, a total of 104 clinical isolates comprising 53 *Escherichia coli* and 51 *Klebsiella pneumoniae from* various clinical samples including urine, stool, sputum, blood, semen, wound and vagina swabs, were tested for ESBL production. The overall prevalence ESBL-producers in our study were 20/104 (19.2%). The results of this findings indicated a growing prevalence level of ESBL producers in our environment; self-medication, easy access to pharmacies, usage of drugs without doctor 'prescription and less stringent antimicrobial policy standards in hospital settings which are common in developing countries, as Nigeria, may be major contributors.

The 19.2% prevalence of this study was consistent and comparable with a recent study conducted in Lagos, Nigeria where the prevalence of ESBL-*Klebsiella pneumoniae* and *Escherichia coli* was found to be 19.5% (Ekpunobi *et al.,* 2024). Similar findings were also reported in other parts of the country such as Nasarawa, Ilorin, Enugu where the prevalence was found to be 22.25%, 26.7% and 26.6% respectively (Barrios *et al.,* 2017; Fadeyi *et al.,* 2018; Husna *et al.,* 2023;). Moreover, reports from some neighboring African countries such as Cameroon, Rwanda and Egypt were somewhat similar (Ameshe *et al.,* 2022; Nkengkana *et al.,* 2023; Elsayed *et al.,* 2024). However, a slightly higher prevalence of 30.0%, 30.5%, 31% and 34% were obtained in Kano State, Anyigba, Lagos state and in other hospitals in six states of Southwestern Nigeria respectively (Olayemi *et al.,* 2020; Akinyemi *et al.,* 2021; Mofolorunsho *et al.,* 2021; Gbolabo *et al.,* 2023). Much higher prevalence rates of 50% in Enugu metropolis (Maduakor *et al.,* 2022), 61% in Anambra South-East (Ezeanya *et al.,* 2017), 74.4% and 43.3% in Ibadan metropolis (Okesola and Oni, 2012; Mojirayo and Adewuyi, 2017) and 51.3% in Ile-Ife (Olowe *et al.,* 2012), all in Nigeria, have also been documented. Conversely, this result was in contrast with previous studies, where rates were reported to be as low as 12.8% in Kaduna State (Olowo-okere *et al.,* 2020), 10.99% in Enugu (Chinedu *et al.,* 2022) and 10% in Southern Nigeria (Ogbolu *et al.,* 2013). These varying prevalence across different studies and geographical regions may be attributed to multiple factors, including differences in sample size, sampling techniques, antibiotic usage patterns and regional infection control practices. Additional research on these contributing variables may provide valuable information on the global epidemiology of *K. pneumoniae and E.coli* that produces ESBL.

CONCLUSION

The findings of this study revealed a growing prevalence of 19.2% of ESBL-producing *Klebsiella pneumoniae and Escherichia coli* with significant resistance observed against Ceftriaxone, Cefotaxime and Ceftazidime. Such prevalence as indicated in this study has called for the need for better diagnostic techniques both in government and private hospitals of Ogbomosho, Nigeria, such as, incorporating ESBL phenotypic detection into routine antimicrobial susceptibility testing, a change in prescription pattern and more importantly, developing a suitable community and hospital antibiotic policies.

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