UMYU Scientifica, Vol. 4 NO. 4, December 2025, Pp 238 – 243
https://doi.org/10.56919/usci.2544.020
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Prevalence and Molecular Detection of ESBL-Producing Gram-Negative Uropathogens Among Pregnant Women in Sokoto Metropolis, Nigeria

[1]*Bello, B.Y., [1]Manga, S.B., [1]A.S Shehu., [2]Maigandi, A., [3]Salisu, H. and [4]Shamsudeen, S.
[1]Faculty of Chemical and Life Sciences, Department of Microbiology, Usmanu DanFodiyo University, Sokoto, Nigeria
[2]Faculty of Agriculture, Department of Agric and Extension, Usman Dan Fodio University, Sokoto, Nigeria
[3]Faculty of Life Sciences, Department of Microbiology, Federal University Gusau, Zamfara, Nigeria
[4]Faculty of Life Sciences, Department of Microbiology, Bayero University, Kano, Nigeria

ABSTRACT
Urinary tract infections (UTIs) during pregnancy pose significant health risks, and the rising prevalence of extended-spectrum β-lactamase (ESBL)-producing organisms further complicates treatment. This study determined the prevalence of ESBL-producing Gram-negative uropathogens and identified associated β-lactamase genes among pregnant women in Sokoto Metropolis. Midstream urine samples were processed using standard microbiological methods. ESBL detection was performed using the Double Disk Synergy Test, and PCR was used to identify *blaTEM*, *blaSHV*, and *blaCTX-M* genes. Of 14 multidrug-resistant isolates, 6 (42.9%) were ESBL producers. *Escherichia coli* (40%) and *Enterobacter* sp. (100%) showed the highest ESBL prevalence. Molecular analysis revealed *blaSHV* in 3 (60%) of the ESBL-positive isolates, while *blaTEM* and *blaCTX-M* were not detected. The predominance of SHV-type ESBLs highlights localized resistance patterns and underscores the need for continuous surveillance and antibiotic stewardship in antenatal care.

ARTICLE HISTORY
Received August 14, 2025
Accepted December 11, 2025
Published December 30, 2025

KEYWORDS
UTI, ESBL, SHV, multidrug resistance

INTRODUCTION

Urinary tract infections (UTIs) remain one of the most common bacterial infections affecting pregnant women worldwide, and they pose significant risks to both maternal and neonatal health. Infections during pregnancy may progress to pyelonephritis, preterm labor, low birth weight, and increased neonatal morbidity (Addis *et al.*, 2021). Over the last decade, the rising trend of antimicrobial resistance among uropathogens has become a major concern in both developed and developing countries. Among these resistant organisms, extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria have emerged as some of the most problematic multidrug-resistant (MDR) pathogens (Shrestha *et al.*, 2016).

ESBLs are plasmid-mediated enzymes that hydrolyze a wide range of β-lactam antibiotics, including penicillins, third-generation cephalosporins, and monobactams, although they are inhibited by β-lactamase inhibitors such as clavulanic acid (Mehmood *et al.*, 2025). The global increase in ESBL-producing bacteria is largely due to their ability to produce β-lactamases such as TEM, SHV, and CTX-M variants, as well as AmpC and OXA-type enzymes, which confer resistance to virtually all β-lactam antibiotics, including the carbapenems (Igweonu, 2024; Uyanga *et al.*, 2020). Importantly, these resistance genes are often carried on plasmids that can be horizontally transferred among bacteria, facilitating the rapid spread of resistance (Zhang *et al.*, 2025).

ESBL-producing uropathogens, particularly *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus species*, and *Pseudomonas aeruginosa*, have been implicated in recurrent and chronic UTIs (Chinyere *et al.*, 2020). Their presence has complicated management options, especially in vulnerable populations such as pregnant women. The situation is further exacerbated by the reliance of many healthcare facilities in Nigeria on dipstick urinalysis rather than urine culture and sensitivity testing, which often results in empirical treatment failure (Onwuezobe & Orok, 2015; Belete, 2020). This study therefore aimed to detect and characterize ESBL-producing Gram-negative uropathogens and to identify the β-lactamase genes (*blaTEM, blaSHV, blaCTX-M*) responsible for resistance among isolates obtained from pregnant women attending antenatal clinics in Sokoto Metropolis, Nigeria.

Correspondence: Baheera Bello Yabo. Faculty of Chemical and Life Sciences, Department of Microbiology, Usmanu DanFodiyo University, Sokoto, Nigeria. ✉ baheerabello2010@gmail.com.
How to cite: Bello, B. Y., Manga, S. B., Shehu, A. S., Maigandi, A., Salisu, H. & Shamsudeen, S. (2025). Prevalence and Molecular Detection of ESBL-Producing Gram-Negative Uropathogens Among Pregnant Women in Sokoto Metropolis, Nigeria. *UMYU Scientifica*, 4(4), 238 – 243. https://doi.org/10.56919/usci.2544.020

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

Midstream urine samples were aseptically collected from pregnant women attending selected antenatal clinics in Sokoto Metropolis. Samples were cultured on CLED and MacConkey agar and incubated at 37°C for 24 hours. Significant bacteriuria was defined as $\geq 10^5$ CFU/mL. Isolates were identified using standard biochemical tests and confirmed by Gram staining.

Screening for ESBL Production

All Gram-negative isolates were subjected to antibiotic susceptibility testing using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Isolates showing resistance to third-generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone) were further screened for ESBL production using the Double Disk Synergy Test (DDST) with amoxicillin-clavulanate (20/10 µg) placed between ceftazidime (30 µg) and cefotaxime (30 µg) disks. Enhancement of the inhibition zone toward the clavulanate disk was interpreted as positive for ESBL production (CLSI, 2022).

Molecular Detection of β-Lactamase Genes

PCR amplification of ESBL resistance genes (*bla*TEM, *bla*CTX-M, *bla*SHV) was performed in a 27 µL reaction mixture containing 1 µL DNA template, 1 µL each of forward and reverse primers, and 12 µL FirePol Master Mix with PCR-grade water. Positive control strains were included for each gene, while sterile water served as a negative control. PCR cycling involved: initial denaturation at 95°C for 7 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing (gene-specific) for 1 min, and extension at 65°C for 8 min, followed by a final extension at 65°C for 16 min (Uyanga *et al.*, 2020).

Agarose gel Electrophoresis

Agarose gel (1%) was prepared by weighing 0.5 gm Agarose in 50 ml 1XTris- Acetate EDTA (TAE) buffer and subjected to heat until the Agarose was completely dissolved and appeared as a clear transparent solution. The Agarose solution was allowed to cool to 50°C and then 1µL of ethidium bromide (0.5µg/ml) dye was added to it. Thereafter, the gel was poured into the gel casting tray with sealed edges and a gel comb was placed into the slots on the tray. The gel was allowed to solidify 30 min and then the comb was gently removed, and the gel slab along with the running tray was submerged carefully into the electrophoresis tank containing 1X TAEbuffer. A total volume of 8 µl amplicon was transferred on a clean Para film and mixed with 2 µl of 6X gel loading dye (Biolabs, UK) and loaded carefully into the wells of Agarose gel. To determine the size of the amplified PCR product, 1Kb DNA ladder (Biolabs, UK) were loaded in the first well. Electrophoresis was performed at 70 V for 1 hour and the mobility were monitored by the migration of the dye in the gel. After appropriate migration, the Agarose gel was visualized under UV trans-illuminator in a Bio-Rad gel documentation device and the results documented (Uyanga *et al.*, 2020).

RESULTS

Out of 14 multidrug-resistant Gram-negative bacterial isolates obtained from pregnant women with urinary tract infections in Sokoto metropolis, 6 (42.9%) were confirmed as extended-spectrum β-lactamase (ESBL) producers, while 8 (57.1%) were non-ESBL producers.

Table 1: Prevalence of ESBL production among the bacterial uropathogens.

Isolates	Number	ESBL producers	Non-ESBL producers
Escherichia coli	5	2 (40.0%)	3 (60.0%)
Klebsiella sp	3	1 (33.3%)	2 (66.7%)
Enterobacter sp	2	2 (100%)	0 (0.0%)
Proteus sp	2	1 (50%)	1 (50%)
P. aeruginosa	2	0 (0.0%)	2 (100%)
Total	**14**	**6 (42.9%)**	**8 (57.1%)**

Table 2: Molecular detection of ESBL genes among phenotypically confirmed ESBL-producing isolates

Gene detected	Amplicon size (bp)	No. of positive isolates	(%)
*bla*CTX-M	~550 bp	0	0.0
*bla*SHV	~450 bp	3	60.0
*bla*TEM	~850 bp	0	0.0

Escherichia coli accounted for the highest number of isolates (n = 5), with 2 (40%) identified as ESBL producers. *Klebsiella* sp. contributed 3 isolates, of which 1 (33.3%) was ESBL-positive. Notably, all *Enterobacter* sp. (n = 2) with 2 (100%) exhibited ESBL production. One out of the 2 *Proteus* sp isolates (50%) was ESBL-positive, while none of the *Pseudomonas aeruginosa* isolates showed ESBL activity (Table 1).

These findings highlight a considerable prevalence of ESBL-producing uropathogens in the study population, underscoring the need for careful antibiotic selection and infection control in antenatal care settings.

PCR amplification was conducted on the 5 phenotypically confirmed ESBL-producing isolates to detect *bla*CTX-M, *bla*SHV and *bla*TEM genes. Amplicons of the expected product sizes were targeted: ~550 bp for *bla*CTX-M, ~450 bp for *bla*SHV, and ~850 bp for *bla*TEM. (Plates 1, 2 and 3). *bla*SHV was the only gene detected, with bands observed in 3 of the 5 isolates tested (60.0%). No amplification was observed for *bla*CTX-M (0/5; 0.0%) or *bla*TEM (0/5; 0.0%) (Table 2).

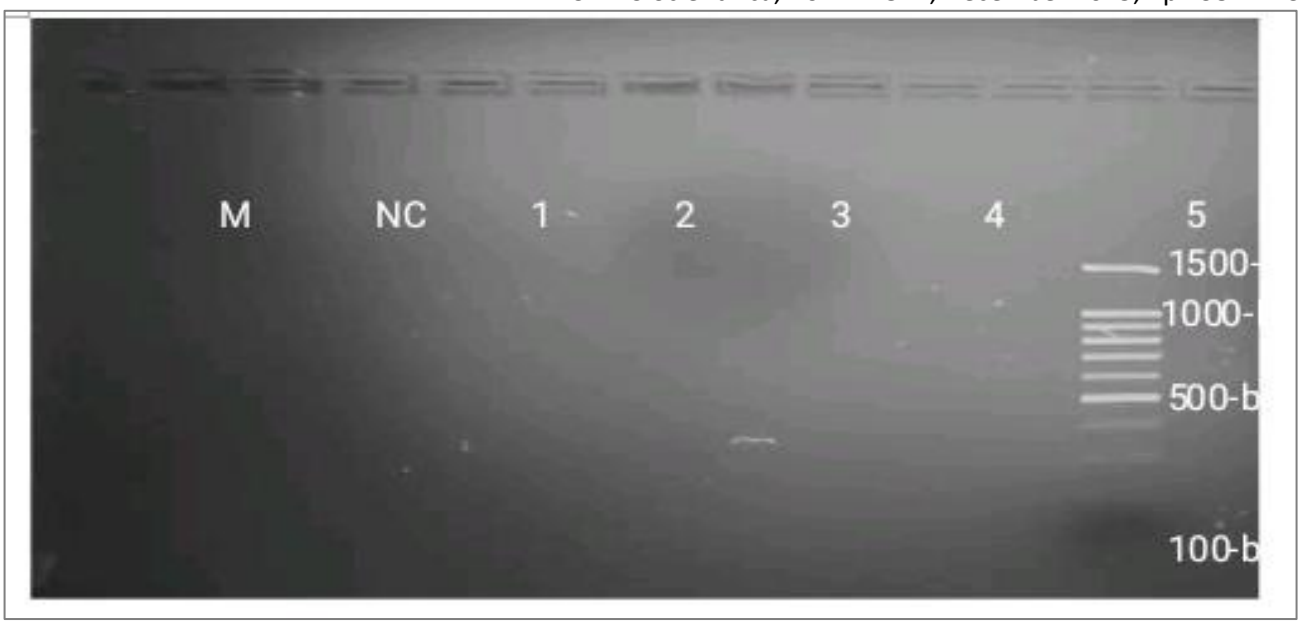

Plate 1: Agarose Gel Electrophoresis of *bla*CTX-M Gene Amplification [Lane M: 100 bp DNA ladder; Lane NC: Negative control; Lanes 1–5: bacterial isolates. Absence of distinct band at~550 bp in all isolates confirm *bla*CTX-M negative.]

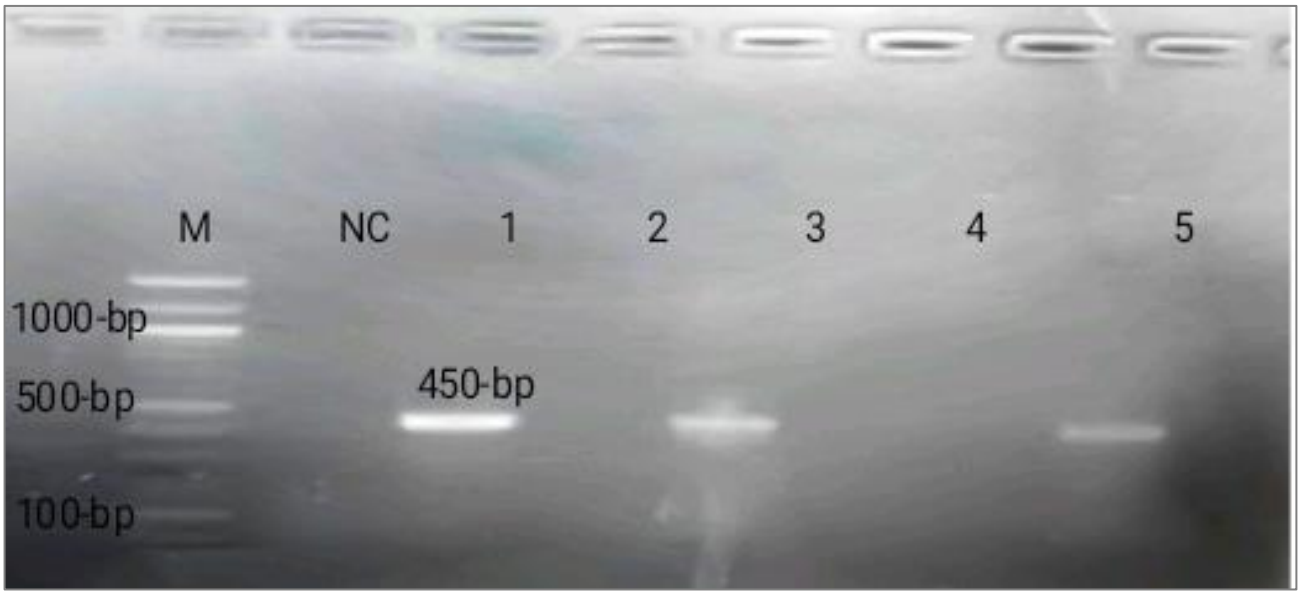

Plate 2: Agarose Gel Electrophoresis of *bla*SHV Gene Amplification [Lane M: 100 bp DNA ladder; Lane NC: Negative control; Lanes 1–5: bacterial isolates. Bands at ~450 bp in Lanes 1, 3, and 5 confirm *bla*SHV positivity.]

DISCUSSION

In this study, 42.9% of multidrug-resistant Gram-negative bacterial isolates from pregnant women with urinary tract infections were identified as extended-spectrum β-lactamase (ESBL) producers. This prevalence is notable and indicates a significant threat to empirical therapy in the study population.

The proportion observed aligns with reports from other regions of Nigeria and sub-Saharan Africa, where ESBL rates among Enterobacteriaceae range from 30–50% (Chinyere *et al.*, 2020; Igweonu, 2024). For instance, a study in Enugu reported 40% ESBL production among *E. coli* and *Klebsiella* isolates (Onwuezobe and Orok, 2015), comparable to our findings of 40% in *E. coli* and 33.3% in *Klebsiella* sp. Similarly, 100% ESBL positivity among *Enterobacter* sp in this study is consistent with reports that this genus frequently harbors plasmid-mediated resistance determinants (Zhang *et al.*, 2025).

The presence of ESBL-producing *Proteus* spp (50%) further highlights the widespread dissemination of resistance mechanisms across diverse Gram-negative pathogens. Conversely, the absence of ESBL production in *Pseudomonas aeruginosa* may reflect its reliance on other resistance mechanisms, such as efflux pumps and AmpC β-lactamases, rather than ESBL enzymes (Belete, 2020).

The 42.9% prevalence of ESBLs observed in this study is higher than reports from some regions of Nigeria (25–35%) but comparable to findings from Ethiopia (41%) and Cameroon (45%) (Tanko *et al.*, 2020; Addis *et al.*, 2021; Nzalie *et al.*, 2016). The differences may be attributed to variations in antibiotic use, infection control practices, and availability of diagnostic facilities.

Plate 3: Agarose Gel Electrophoresis of *bla*TEM Gene Amplification [Lane M: 100 bp DNA ladder; Lane NC: Negative control; Lanes 1–5: bacterial isolates, at ~850 bp no bands observed, indicating absence of the *bla*TEM gene]

The molecular results of this study showed that *blaSHV* was the only ESBL gene detected, present in 60.0% of isolates tested, while neither *blaCTX-M* nor *blaTEM* was identified. This finding indicates that SHV-type β-lactamases are the predominant ESBL determinant among Gram-negative uropathogens from pregnant women in Sokoto metropolis.

This observation contrasts with global patterns, where *blaCTX-M* is reported as the most frequently detected ESBL gene (Bevan *et al*., 2017; Castanheira *et al.,* 2021). In Nigeria, Akinyemi *et al.* (2021) documented CTX-M as the most common ESBL type in Lagos, while Uyanga e*t al.* (2020) reported widespread co-existence of CTX-M, SHV, and TEM among *E. coli, K. pneumoniae, E. cloacae* isolates in South-south Nigeria. The absence of CTX-M in the present study may reflect geographical variation in gene distribution, differences in antibiotic usage practices, or limitations related to the relatively small number of isolates tested.

The predominance of *blaSHV* in this study aligns with earlier reports linking SHV enzymes to *Klebsiella pneumoniae* and other *Enterobacteriaceae*. The plasmid-mediated nature of SHV facilitates horizontal gene transfer, ensuring persistence in bacterial populations and enhancing the spread of resistance (Castanheira *et al*., 2021). Similar findings were reported in southeastern Nigeria, where Chioma *et al.* (2019) also documented the presence of SHV genes in uropathogenic *E. coli* and *K. pneumoniae* from pregnant women, emphasizing the continued clinical importance of SHV enzymes in maternal UTIs.

The absence of *blaTEM* in this study further supports the global trend of declining TEM prevalence as CTX-M and SHV have become more dominant in clinical isolates (Onyango *et al*., 2018; Askari *et al*., 2024). However, studies from other regions have reported different patterns. For example, Maina *et al.* (2012) in Kenya identified CTX-M as the most predominant gene in urinary isolates, while Muriuki *et al.* (2022) also documented high prevalence of CTX-M (95.6%) and TEM (95.6%) in comparison to SHV (21.7%). Similarly, Ghaddar *et al.* (2020) in Lebanon reported CTX-M (90.7%) as the most predominant ESBL gene, followed by TEM (88.4%) and SHV (44.2%). In Somalia, Hussein *et al.* (2021) found TEM (71.4%) and CTX-M (66.7%) to be the most frequent, with SHV detected in only 3.2% of isolates. In Iran, Amiri *et al.* (2015) reported TEM (66.7%) as the most common ESBL gene, followed by CTX-M (33.3%).

CONCLUSION

Among the 14 multidrug-resistant Gram-negative isolates, 6 (42.9%) were confirmed as extended-spectrum β-lactamase (ESBL) producers, with *E. coli* and *Enterobacter* spp. showing the highest ESBL prevalence. PCR amplification of the ESBL genes revealed the presence of the *blaSHV* gene in 3 (60%) of 5 tested isolates; *blaTEM* and *blaCTX-M* were not detected.

REFERENCES

Addis, T., Mekonnen, Y., Ayenew, Z., Fentaw, S., & Biazin, H. (2021). Bacterial uropathogens and burden of antimicrobial resistance pattern in urine specimens referred to Ethiopian Public Health Institute. *PloS One, 16*(11), e0259602. [Crossref]

Akinyemi, K. O., Abegunrin, R. O., Iwalokun, B. A., Fakorede, C. O., Makarewicz, O., Neubauer, H., & Wareth, G. (2021). The emergence of *Klebsiella pneumoniae* with reduced susceptibility against third generation cephalosporins and carbapenems in Lagos Hospitals, Nigeria. *Antibiotics, 10*(2), 142. [Crossref]

Amiri, M., Lavasani, Z., Norouzirad, R., Najibpour, R., Mohamadpour, M., & Nikpoor, A. R. (2015). Prevalence of urinary tract infection among pregnant women and its complications in their newborns during the birth in the hospitals of Dezful City, Iran, 2012-2013. *Iranian Red Crescent Medical Journal, 17*(8), e26946. [Crossref]

Askari, S., Badouei, M. A., Aflakian, F., & Hashemitabar, G. (2024). Phenotypic and genotypic evaluation of extended-spectrum β-lactamases (bla TEM, bla SHV, bla CTX-M, bla OXA) in *Escherichia coli* isolated from children with diarrhea. *Biologia, 79*(11), 3433–3439. [Crossref]

Belete, M. A. (2020). Bacterial profile and ESBL screening of urinary tract infection among asymptomatic and symptomatic pregnant women attending antenatal care of Northeastern Ethiopia Region. *Infection and Drug Resistance, 13*, 2579–2592. [Crossref]

Bevan, E. R., Jones, A. M., & Hawkey, P. M. (2017). Global epidemiology of CTX-M β-lactamases: Temporal and geographical shifts in genotype. *Journal of Antimicrobial Chemotherapy, 72*(8), 2145–2155. [Crossref]

Castanheira, M., Simner, P. J., & Bradford, P. A. (2021). Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. *JAC-Antimicrobial Resistance, 3*(3), dlab092. [Crossref]

Chinyere, O. M., Nwankwo, E. O., & Nsofor, C. A. (2020). Prevalence of multidrug-resistant and extended-spectrum β-lactamase-producing Enterobacteriaceae in urinary tract infections among pregnant women in southeastern Nigeria. *Journal of Global Antimicrobial Resistance, 22*, 134–140. [Crossref]

Chioma, O. P., Martina, C. A., & Emmanuel, A. E. (2019). ESBL mediated antimicrobial nonsusceptibility of uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* isolates from pregnant women in Nnewi, Nigeria. *Journal of Advances in Microbiology, 18*(2), 1–3. [Crossref]

Clinical and Laboratory Standards Institute. (2022). *Performance standards for antimicrobial susceptibility testing* (32nd ed., CLSI supplement M100).

Ghaddar, N., Anastasiadis, E., Halimeh, R., Ghaddar, A., Matar, G. M., & Abou Fayad, A. (2020). Phenotypic and genotypic characterization of extended-spectrum β-lactamases produced by *Escherichia coli* colonizing pregnant women. *Infectious Diseases in Obstetrics and Gynecology, 2020*, 4190306. [Crossref]

Hussein, E. F., Ameen, J. A., & Yassen, S. H. (2021). Study the antibiotics activity against *Escherichia coli* isolated from urine samples of pregnant women with urinary tract infection. *International Journal of Pharmaceutical Research, 13*(1), 1368–1372. [Crossref]

Igweonu, C. F. (2024). Molecular characterization of antibiotic resistance genes in multidrug-resistant *Klebsiella pneumoniae* clinical isolates. *International Journal of Engineering Technology Research and Management, 8*(8), 241–249.

Iregbu, K. C., Nwajiobi-Princewill, P. I., & Iwuafor, A. A. (2016). Urinary tract infections in pregnancy in Abuja, Nigeria: Bacteriological profile and antimicrobial susceptibility pattern. *The Nigerian Postgraduate Medical Journal, 23*(4), 223–227. [Crossref]

Iroha, I. R., Ozor, H. O., Moses, I. B., Onuora, A. L., & Kalu, A. C. (2020). Antimicrobial activity of ethyl acetate and ethanol seed extracts of *Buchholzia coriacea* (Wonderful Kola) against multidrug-resistant clinical isolates of *Salmonella* species and *Escherichia coli*. *Archives of Clinical Microbiology, 11*(3), 109–115. [Crossref]

Maina, D., Revathi, G., Kariuki, S., & Ozwara, H. (2012). Genotypes and cephalosporin susceptibility in extended-spectrum β-lactamase producing Enterobacteriaceae in the community. *The Journal of Infection in Developing Countries, 6*(6), 470–477. [Crossref]

Mehmood, A., Arooj, I., Sultan, R., Javeed, M., & Javaid, A. (2025). Antibiotic sensitivity and ESBL prevalence among Gram-negative bacteria in a tertiary care hospital. *Lahore Garrison University Journal of Life Sciences, 9*(1), 1–11. [Crossref]

Muriuki, C. W., Ogonda, L. A., Kyanya, C., Matano, D., Masakhwe, C., & Odoyo, E. (2022). Phenotypic and genotypic characteristics of uropathogenic *Escherichia coli* isolates from Kenya. *Microbial Drug Resistance, 28*(1), 31–38. [Crossref]

Nzalie, R. N. T., Gonsu, H. K., & Koulla-Shiro, S. (2016). Bacterial etiology and antibiotic resistance profile of community-acquired urinary tract infections in a Cameroonian city. *International Journal of Microbiology, 2016*, 3240268. [Crossref]

Onwuezobe, I. A., & Orok, F. E. (2015). The bacterial isolates and plasmid profiles of extended spectrum beta-lactamases producers causing urinary tract infections among pregnant women in Uyo, Nigeria. *Journal of Biosciences and Medicines, 3*(7), 25–30. [Crossref]

Onyango, H. A., Ngugu, C., Maina, J., & Kiiru, J. (2018). Urinary tract infection among pregnant women at Pumwani Maternity Hospital, Nairobi, Kenya: Bacterial etiologic agents, antimicrobial susceptibility profiles and associated risk factors. *Advances in Microbiology, 8*(2), 175–187. [Crossref]

Shrestha, A., Manandhar, S., Pokharel, P., Panthi, P., & Chaudhary, D. K. (2016). Prevalence of extended spectrum beta-lactamase (ESBL) producing multidrug resistance Gram-negative isolates causing urinary tract infection. *EC Microbiology, 4*(5), 749–755.

Tanko, N., Bolaji, R. O., Olayinka, A. T., & Olayinka, B. O. (2020). A systematic review on the prevalence of extended-spectrum beta lactamase-producing Gram-negative bacteria in Nigeria. *Journal of Global Antimicrobial Resistance, 22*, 488–496. [Crossref]

Ugwu, M. C., Shariff, M., Nnajide, C. M., Beri, K., Okezie, U. M., & Iroha, I. R. (2020). Phenotypic and molecular characterization of β-lactamases among enterobacterial uropathogens in Southeastern Nigeria. *Canadian Journal of Infectious*

Diseases and Medical Microbiology, 2020, 5843904. [Crossref]

Uyanga, F. Z., Ekundayo, E. O., Nwankwo, E. O., & Ibanga, I. A. (2020). Molecular characterization of extended spectrum beta-lactamase from *Enterobacter cloacae*, *E. coli* and *Klebsiella pneumoniae* from pregnant women in South-south Nigeria. *International Journal of Microbiology and Biotechnology, 5*(2), 48–54. [Crossref]

Zhang, S., Yang, J., Abbas, M., Yang, Q., Li, Q., Liu, M., & Cheng, A. (2025). Threats across boundaries: The spread of ESBL-positive Enterobacteriaceae bacteria and its challenge to the "one health" concept. *Frontiers in Microbiology, 16*, 1496716. [Crossref]