Prevalence of Extended Spectrum β-Lactamases-Producing *Escherichia coli* Isolated from Clinical Samples and Their Antibiotic Resistance Pattern

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ABSTRACT

Introduction: Infections caused by ESBL-producing bacteria are a growing concern worldwide, especially in developing countries like Nigeria. Hence, this study aimed to isolate, screen, and identify ESBL-producing *Escherichia coli* from clinical samples and analyze their antibiotic resistance patterns. **Methods:** 200 clinical samples were collected, consisting of 60 stool, 88 urine, and 52 wound pussamples. We used the pour-plate method on MaCconkey agar (MAC) for isolation. After suspected *E. coli* was isolated, we sub-cultured it on eosin methylene blue (EMB) agar. To confirm *E. coli* identification, we used cultural and biochemical assays. Disc and double disc diffusion methods were employed to screen and confirm ESBL-producing *E. coli*. Antimicrobial susceptibility testing was conducted by disc diffusion technique, and the results were interpreted using CLSI standards. **Results:** A total of 47 *E. coli* isolates were obtained, with 48.5% of the isolates originating from urine samples. These isolates were among six different genera of bacteria. Among the *E. coli* isolates, 16 were confirmed to be ESBL producers. The ESBL-producing *E. coli* demonstrated high resistance to amoxicillin-clavulanic acid (87.5%), ampicillin (75.0%), and cefotaxime (50.0%). Before plasmid curing, the bacteria demonstrated a high susceptibility to chloramphenicol (81.3%) and amikacin (56.3%). However, varying antibiotic resistance and susceptibility degrees were observed after plasmid curing. **Conclusion:** ESBL-producing *E. coli* showed a high resistance level (34.0%) against most discs used. However, chloramphenicol and amikacin showed promise as potential treatments for ESBL-producing *E. coli* infections. In addition, it is recommended that clinical laboratories should include routine ESBL detection methods for ongoing surveillance of antibiotic-resistant isolates. This will serve as a helpful guide for empirically treating bacterial infections.

INTRODUCTION

Microbial resistance represents a significant global health challenge, particularly in sub-Saharan Africa and South Asia [1]. This occurs when bacteria, once susceptible to antimicrobial agents, develop a resistance mechanism in the presence of a specific antibiotic, leading to increased difficulty in treating infections. In 2019, a report highlighted that bacterial antimicrobial resistance led to approximately 50,000 deaths in sub-Saharan Africa [1].

The production of extended-spectrum β-lactamases is one of the ways bacteria have developed resistance to antimicrobial agents. These enzymes confer resistance to β-lactam antibiotics, the primary target of antibiotic resistance in *Enterobacteriaceae*. Different β-lactamases have been identified, including penicillinases, extended-spectrum β-lactamases (ESBLs), cephalosporinases (AmpC), Metallo-β-lactamases (MBLs), and carbapenemases (KPCs) [2]. Gram-negative Enterobacteriaceae and other bacterial groups produce these enzymes and can break down extended-spectrum antibiotics such as cephalosporins, penicillins, and monobactam [3]. The genes responsible for extended-spectrum β-lactamases are often carried on plasmids and have diverse structures that evolve quickly, making extended β-lactamase-producing bacteria the primary cause of hospital-acquired infections [4].

Escherichia coli, a member of the *Enterobacteriaceae* family, is notorious for causing nosocomial infections and is frequently resistant to β-lactams antibiotics [3]. The excessive use of antibiotics has been linked to an increase in the prevalence of β-lactamase-producing bacteria, such as *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, within hospital environments. These bacteria are responsible for multiple forms of antimicrobial resistance [5].

In Africa, the absence of a locally developed clinical protocol to determine when to use the appropriate antimicrobial agents is a significant obstacle to curbing

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the emergence and spreading of resistance in microorganisms, making quality healthcare delivery herculean task [6]. In Nigeria, there have been widespread reports of antimicrobial resistance to commonly available antibiotics, such as tetracyclines, penicillins, aminoglycosides, and clotrimazole [7]. A multi-regional study involving five of the six geopolitical zones in Nigeria also reported high levels of antimicrobial resistance among *E. coli* isolates to penicillins, cephalosporins, streptomycin, chloramphenicol, tetracyclines and cotrimoxazole [8].

This study aims to isolate and identify *E. coli* strains from clinical samples from patients at the Federal Medical Centre, Yola, Adamawa State, Nigeria. The study will screen these samples for *E. coli* strains that produce extended-spectrum β-lactamases and determine their antimicrobial resistance patterns.

MATERIAL AND METHODS

Two hundred clinical specimens were collected from patients who attended the Federal Medical Center (FMC) in Yola, Adamawa State, Nigeria. The samples included 60 stools and 88 urine specimens collected using sterile universal bottles. Additionally, 52 wound pus samples were collected using sterile swab sticks, placed in nutrient broth, and incubated at 37 ^{0}C [9]. The stool and urine samples were immediately placed in ice packs and transported to the Microbiology Department of Modibbo Adama University's postgraduate laboratory in Yola, Adamawa State, Nigeria, for analysis.

Ethical approval was obtained from the Management and Ethical Committee of the Federal Medical Center Yola (reference FMCY/HREC/21/124), and informed consent was obtained from all patients who provided samples for this study.

Isolation and screening of bacterial isolates for *E. coli*. Ten-fold serial dilution of the stool, urine, and overnight growth of wound pus samples was carried out using the method described by Cullen and MacIntyre [10]. 0.1 mL of each sample at a dilution of 10^{-4} was dispensed into sterile Petri dishes and covered with molten, cooled MacConkey agar. We gently rocked the plates to ensure an even distribution of the samples and then incubated them at 37 ^{0}C for 24 h. After 24 hours of incubation, we sub-cultured the pinkish colonies by streaking them on EMB agar and incubating them at 37 ^{0}C. Colonies that displayed a metallic greenish sheen on EMB agar were suspected to be *E. coli and* were subsequently confirmed using biochemical tests [11]. We incubated all confirmed *E. coli* isolates on a nutrient agar slant at 37 °C. After 24 h, we stored the isolates in a refrigerator (Haier Thermocool) at 4 °C.

Biochemical identification of *E. coli*. We subjected all isolates presumptively identified as *E. coli* based on cultural characteristics to biochemical tests to confirm their identity. The tests we conducted to verify their identity included Gram staining, indole production, methyl red, Voges-Proskauer, citrate, sugar fermentation, triple sugar iron agar, and motility tests using the methods described by Fawole and Oso [11].

Screening and confirmation of extended-spectrum beta-lactamase *E. coli*. Using the standard disk diffusion method, we tested the confirmed *E. coli* isolates for their ability to produce ESBLs. We used disks containing ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg) (Oxoid, UK) [12]. We confirmed the suspected ESBL-producing *E. coli* using the double-disk synergy (DDS) method [12].

Antimicrobial susceptibility testing of ESBL-producing *E. coli*. We performed antimicrobial susceptibility testing using the Kirby–Bauer disc-diffusion technique on Mueller–Hinton agar as described by Hudzicki [13]. The antimicrobial discs included amoxicillin/clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µ g), ampicillin (10 µg), amikacin (30 µg), cefpodoxime (30 µg), and chloramphenicol (30 µg) [13].

We measured the zones of inhibition of each antimicrobial disc in millimeters using a metric ruler. We interpreted them as resistant (R), intermediate (I), or susceptible (S) based on the interpretative chart provided by Hudzicki [13].

Plasmid curing of ESBL-producing *E. coli*. To determine the location of the drug resistance marker(s) (plasmid-borne or chromosomal), we performed plasmid curing. We eliminated the resistant genes on the plasmids of the bacterial isolates using a sub-inhibitory concentration of 1 g per 100 mL of sodium dodecyl sulfate (SDS) as described by others [14]. We prepared nutrient broth with 1.0 g per 100 mL of SDS and inoculated it with ESBL-producing *E. coli* isolates. We incubated the broth at 37°C for 24 h. We used a sterile Pasteur pipette to place two drops of homogenized broth culture onto Mueller Hinton agar plates. Then, we spread a lawn of the inoculum evenly on the surface of the agar using a sterile bent glass rod. We used sterile forceps to place antimicrobial discs (previously used in antimicrobial susceptibility testing) onto the agar. We incubated the plates aerobically at 37°C for 24 h and observed and measured the inhibition zones using a metric ruler. The absence of an inhibition zone on Mueller Hinton agar indicated plasmid-mediated resistance (plasmid cured). In contrast, a zone of inhibition on Mueller Hinton agar showed chromosome-mediated resistance (non-cured plasmid).

RESULTS

Isolation and screening for *E. coli*. We recovered 72 isolates belonging to six genera in the *Enterobacteriaceae* family from the 200 samples used in this study. Based on their cultural characteristics on MacConkey and EMB

agars, we obtained 47 *E. coli* isolates through screening. Out of the total *E. coli* isolates, 28 (59.6 %) were recovered from stool samples, 11 (23.6 %) from urine samples, and eight (17.0 %) from wound swabs (Table 1). Among the six bacterial genera isolated, *E. coli* was the most prevalent, accounting for 47 (65.3 %) isolates.

Yersinia sp. had the lowest prevalence, with only 1.0 (1.4 %) isolate recovered (Table 2). Other isolated and tentatively identified genera included *Klebsiella* sp., *Proteus* sp., *Morganella* sp., *Yersinia* sp., and *Citrobacter* sp.

Table 1. Prevalence of *E. coli* isolates from different clinical samples

Specimens	No.	*E. coli* (%)
Stool	60	28 (59.6)
Urine	88	11 (23.4)
Wound Swab	52	8 (17.0)
Total	200	47

Table 2. Biochemical characteristics of *E. coli* and other isolated bacteria

Mot	Cit	Ind	TSIA		H₂S	Gas	VP	MR	Man	Glu	Suc	GR	Isolate identity	Frequency (%)
			Slope	Butt										
+	-	+	Y	Y	-	+	-	+	+	+	+	-	*E. coli*	47 (65.3)
-	+	-	Y	Y	-	+	+	-	+	+	+	-	*K. pneumonia*	7 (9.7)
+	-	+	R	Y	+	-	-	NA	NA	NA	NA	-	*P. vulgaris*	9 (12.5)
+	+	-	R	Y	-	+	-	+	+	+	NA	-	*Citrobacter* sp.	5 (6.9)
+	-	+	R	Y	-	+	NA	NA	NA	NA	NA	-	*Morganella* sp.	3 (4.2)
+	-	-	Y	R	-	-	NA	NA	NA	NA	NA	-	*Yersinia* sp.	1 (1.4)

Key: Cit = Citrate, Mot =Mobility, Ind = Indole, H₂S = Hydrogen sulphide (Blackening) R = red-pink (alkaline reaction), Y = Yellow (acid reaction), VP = Voges-Proskauer, MR = methyl red, Man = mannitol, Glu = glucose, Suc = sucrose, GR = Gram reaction, NA = Not applicable, + = positive, - = negative

Screening and confirmation of Extended Spectrum Beta-Lactamase *E. coli*. We subjected 47 *E. coli* isolates to the disc diffusion method to determine their ESBL production. Among them, 19 (40.4 %) *E. coli* isolates were found to be positive as ESBL producers (Fig. 1). To confirm the 19 ESBL-producing *E. coli* isolates; we used the double disc synergy technique with antibiotic discs containing cefotaxime (30 µg), amoxicillin+clavulanic acid (20 µg+10 µg) and ceftazidime (30 µg). However, out of the 19 ESBL-producing *E. coli* isolates identified earlier, only 16 (34.0 %) were confirmed to be positive based on the improved zone of inhibition of more than 5.0 mm observed against the antibiotic discs used (Fig. 2).
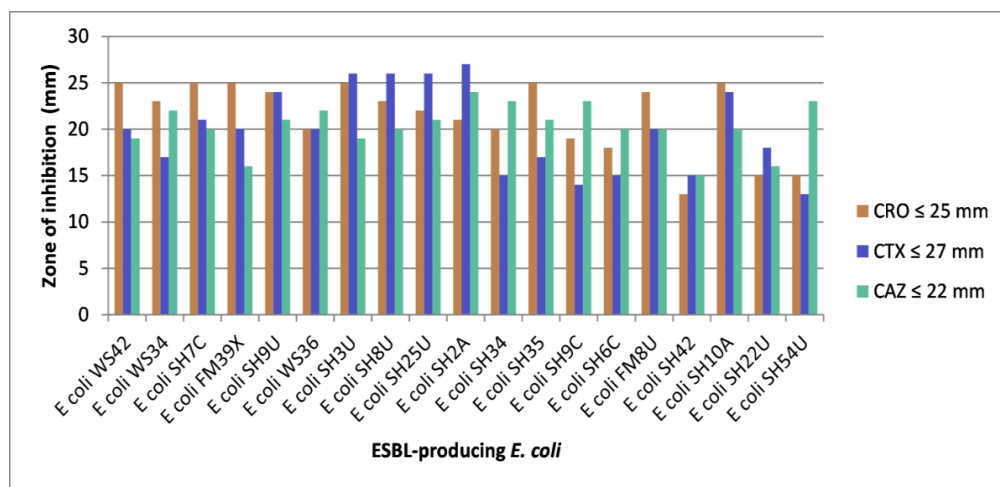

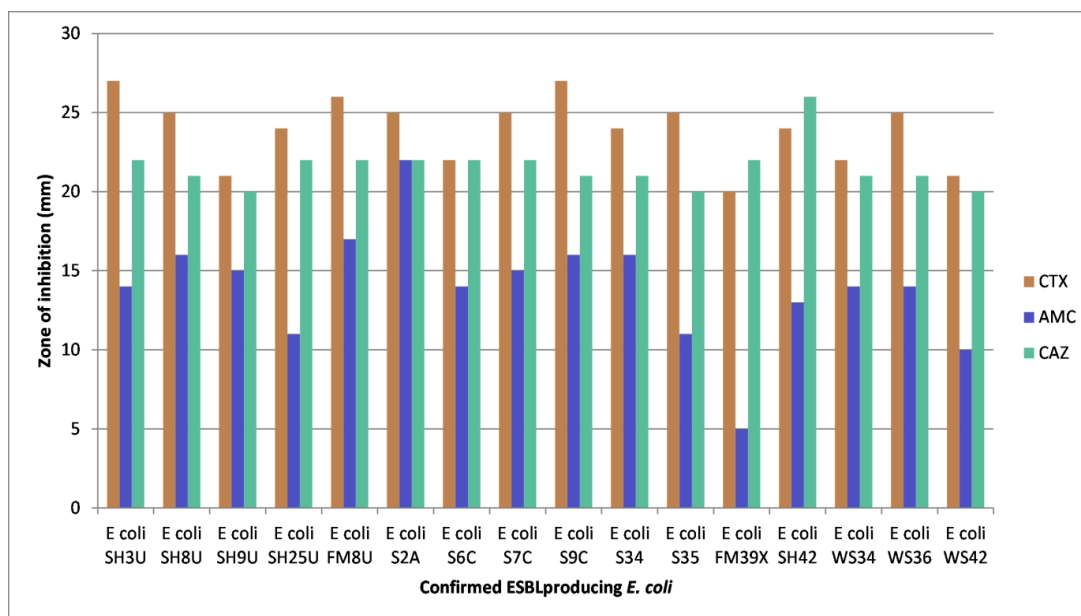**Fig. 1.** ESBL-producing *E. coli* isolates

Fig. 2. Confirmed ESBL-producing *E. coli* isolates

Antibiotic susceptibility pattern of ESBL-producing *E. coli* before and after plasmid curing. Before plasmid curing, we tested all 19 ESBL-producing *E. coli* isolates for antibiotic resistance. o determine their susceptibility, we used eight different antibiotics, including ceftazidime, cefotaxime, ceftriaxone, chloramphenicol, amoxicillin/clavulanic acid, cefpodoxime, amikacin, and ampicillin. The ESBL-producing *E. coli* exhibited the highest resistance to amoxicillin+clavulanic acid (87.5 %) and ampicillin (75.0 %), while their susceptibility was highest to chloramphenicol (81.3 %) (Fig. 3).
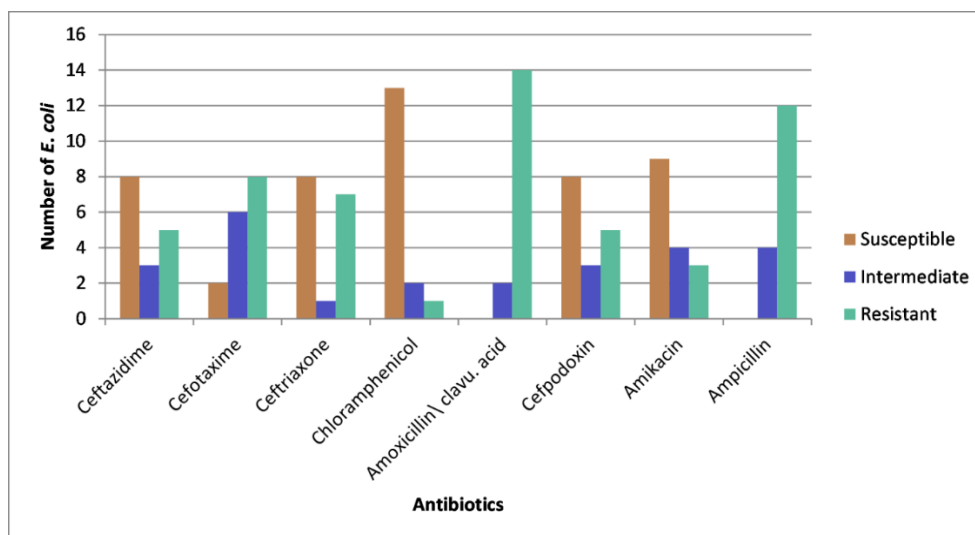

Fig. 3. Antibiotics susceptibility profile of ESBL-producing *E. coli* isolates before plasmid curing.

After plasmid curing (elimination) of *E. coli*, we observed changes in their resistance patterns. Some isolates lost their resistance and became susceptible to ceftriaxone, ceftazidime, and amoxicillin+clavulanic acid (Fig. 4). We also observed some reductions in resistance of ESBL-producing *E. coli* towards other antibiotics such as ampicillin, amikacin, cefpodoxime, and cefotaxime (Fig. 4).

DISCUSSION

ESBL-producing *E. coli* and other members of *Enterobacteriaceae* have caused severe health and economic issues worldwide. In this study, we isolated 72 bacteria from the family *Enterobacteriaceae*. The isolated bacteria were classified into six genera: *Escherichia*, *Klebsiella*, *Citrobacter*, *Morganella,* and *Yersinia*. In this study, *E. coli* was the most prevalent species, consistent with the findings of Mirkalantari *et al*. [15], who

investigated urine samples. However, studies have reported conflicting results regarding the predominant bacteria isolated from various clinical samples, with some identifying *E. coli* or *K. pneumoniae* as the most common species [16, 17]. In contrast, others have reported *Enterobacter* as the most prevalent genus [18]. Of the total *E. coli* isolates, stool samples accounted for approximately 60 %, followed by 24.4 % from urine and 17.0 % from wound swabs (Table 1). Enteric bacteria are a common part of the bacterial flora of humans and other animals and are generally present in the hospital and community settings [16].
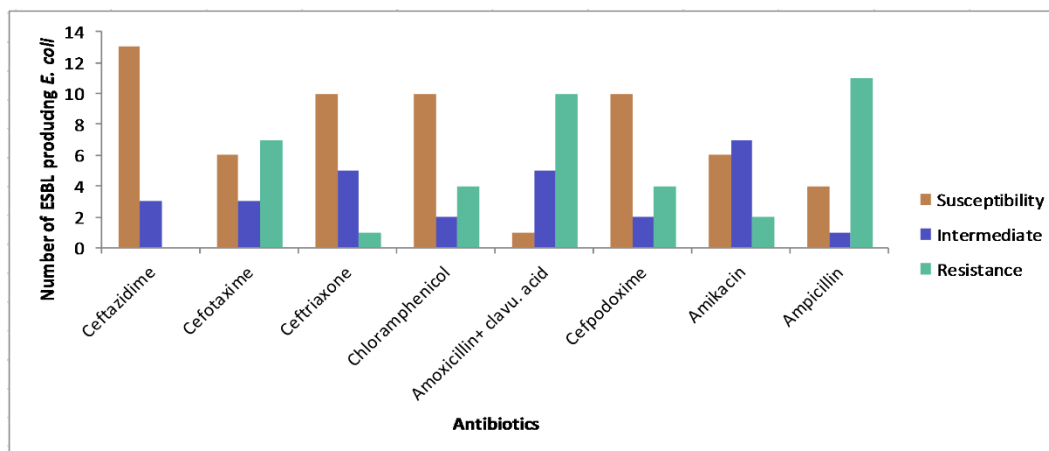

Fig. 4. Antibiotic resistance pattern of ESBL-producing *E. coli* isolates after plasmid curing.

The identification of *E. coli* isolates was based on their cultural characteristics on MacConkey and EMB agars. The pinkish and metallic greenish sheen observed is consistent with previous reports by other researchers [19, 20]. The appearance of *E. coli* on MacConkey and EMB agars is a well-known characteristic used for its tentative identification. Biochemically, all *E. coli* isolates in this study tested positive for indole, methyl red, mannitol, glucose, and sucrose but negative in reactions to citrate, hydrogen sulfide, Voges-Proskauer, and they were Gram-negative (Table 2). These biochemical traits for identification were similar to those reported by Mahe *et al*. [21].

The screening of the 47 *E. coli* isolates for ESBL production showed that 19 (40.4%) were positive, with 16 confirmed as ESBL-producing *E. coli*. The prevalence of ESBL-producing *E. coli* in this study was 34.0%, which is consistent with the 33.0% prevalence reported in 2017 [22] but higher than the 18.0% reported in 2016 [23]. The increased prevalence of ESBL-producing *E. coli* observed in this study could be attributed to poor environmental conditions within the hospital and inappropriate use of antibiotics by patients [24].

The findings of this study revealed that ESBL-producing *E. coli* isolates were more prevalent in urine specimens, followed by wound swab specimens and stool specimens. This is consistent with earlier reports by Shashwati and Kiran [25] and Kateregga *et al*. [26], which reported 52.28 % and 47.4 % prevalence in urine and wound samples, respectively. The distribution of ESBL-producing bacteria across different specimens can vary, and this variation may be attributed to differences in the number of study participants and their exposure to antibiotics.

In this study, the antibiotic susceptibility pattern before plasmid curing revealed the highest resistance levels among ESBL-producing *E. coli* isolates to amoxicillin+clavulanic acid, followed by ampicillin and cefotaxime. After plasmid curing, there was a slight reduction in the resistance to these antibiotics (Fig. 4). Our findings in this study are consistent with an earlier report of Buckner *et al*. [27], which described the use of sodium dodecyl sulfate for treating bacteria to eliminate plasmids inside them. These observations show that the antibiotic-resistance treatment options usually available and prescribed against nosocomial pathogenic bacteria, especially ESBL-producing *E. coli*, are becoming less effective. Likewise, the resistance profile of ESBL-producing *E. coli* to third-generation cephalosporins observed in this study was lower than that reported in a similar study [3], which found 82.8%-90.0% resistance. This indicates that the third-generation cephalosporin antibiotics, which were once effective, are no longer viable options for treating ESBL-producing *E. coli* infections due to widespread resistance [28]. ESBL-producing bacteria often exhibit co-resistance to multiple classes of antibiotics, which reduces the range of effective treatment options for infections caused by these bacteria [4].

Furthermore, chloramphenicol was found to be the most effective against the ESBL-producing *E. coli* isolates used in this study, followed by amikacin (Fig. 3). Our findings are consistent with previous reports by Giwa *et al*. (2018) and Hussain *et al*. (2012), which also showed high susceptibility of *E. coli* to amikacin and chloramphenicol, respectively [29, 30]. Their result was

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higher than ours, likely due to the more significant number of test strains, but it confirms that amikacin is against ESBL-producing *E. coli*. While carbapenems have been the preferred antibiotics for treating ESBL-producing pathogens, their high cost makes them unaffordable for many people in developing countries [31]. This study also found that amikacin was effective against ESBL-producing *E. coli*. This is consistent with the findings of Cho *et al.* (2016), who reported similar observations in treating ESBL-producing UTI-causing microbes [31]. Generally, amikacin may be a valuable option for treating infections caused by ESBL-producing *E. coli* and other enteric pathogenic bacteria [31, 32]. It is a more affordable choice for those with limited resources. However, its toxicity is still debatable, mainly when used in high dosages [31].

To summarize, we isolated 72 Gram-negative enteric bacteria from six different genera in different clinical specimens. Among these, *E. coli* was the most prevalent, with over 40% of the isolates confirmed to be ESBL producers. We observed high resistance levels to amoxicillin+clavulanic acid, and ampicillin was reported. However, chloramphenicol and amikacin were more effective against the ESBL-producing *E. coli* isolates studied. Despite this, moderate resistance was noted among the cephalosporin antibiotics, making them less suitable for treating nosocomial infections caused by ESBL-producing *E. coli*. The widespread occurrence of ESBL-producing bacterial infections could be attributed to the indiscriminate use of antibiotics, which results from selective pressure of resistant genes, an unhygienic hospital environment, and the transfer of resistant genes among members of the *Enterobacteriaceae* family. Therefore, it is necessary to conduct regular surveillance of antimicrobial resistance in hospitals and patients to identify the most effective antibiotics for treatment.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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