

Beta-lactam resistance and phenotypic detection of extended-spectrum beta-lactamase in Enterobacteriaceae isolated from community-acquired urinary tract infections

Abstract

Introduction : Urinary infections are found in both sexes and strike at all ages, occupying a prominent place in nephrological pathology. The biggest problem is that a pathology with a wide range of antibiotics and other hygiene measures that can remedy it, remains so frequent with sometimes serious complications that can compromise the vital prognosis.

Objectives : determine the germs of urinary infections; determine the antibiogram profile of enterobacteria in urinary infections

Methodology : This is a cross-sectional study with an analytical aim of enterobacteria isolated from urinary infections from samples from patients of all ages from January 1, 2020 to October 21, 2022.

Results: the isolated enterobacteria strains presented high rates of resistance, i.e. 92.61% for Ampicillin, 47.94% for cefadroxil, 45.14% for cefuroxime and 46.46% for cefotaxime, 72.86% for amoxicillin and 40.44% for amoxicillin + clavulanic acid. Qualitative detection of extended spectrum Beta Lactamase was generally evaluated at 24.8% with peaks for Citrobacter sp, Klebsiella sp and E coli. For the latter, cross-resistance to quinolones was evaluated in proportions ranging from 49.45% to 85.51%.

Conclusion: This study shows that the level of resistance of enterobacteria to beta-lactams is very high. We have observed co-resistance between beta-lactams and quinolones, antibiotics commonly used against Gram-Negative bacteria. This observation requires an improvement in the antibiotic management policy.

1. INTRODUCTION

Antibiotic resistance is increasing worldwide and is associated with serious morbidity and mortality, increased healthcare costs, and reduced gross domestic product (GDP) globally[1]. Beta-lactams are preferred due to their clinical effectiveness and safety due to their highly selective toxicity [2]. Beta-lactam resistance in Enterobacteriaceae and other Gram-negative organisms is mainly mediated by beta-lactamases [3]. Beta-lactamases are enzymes that catalyze the beta-lactam hydrolysis ring, leading to inactivation of antimicrobials and preventing it from being active against enzymes responsible for bacterial cell wall synthesis [4] .

Extended-spectrum β -lactamases (ESBLs) are a group of diverse, complex, and rapidly evolving plasmid-mediated enzymes that today pose a major therapeutic challenge in the treatment of hospitalized and community patients. Infections caused by expanded beta-lactamase producers range from simple urinary tract infections to life-threatening sepsis[5]. Currently, organizations such as the Institute for Clinical and Laboratory Standards (formerly the National Committee for Clinical Laboratory Standards) provide guidelines for the detection of ESBLs in *Klebsiella pneumoniae*, *K. oxytoca*, *Escherichia coli*, and *Proteus mirabilis*[6]. All methods for detecting extended beta-lactamases have in common the general principle

that the activity of extended-spectrum cephalosporins against extended beta-lactamase-producing organisms will be enhanced by the presence of clavulanic acid[7].

Expanded beta-lactamases represent an impressive example of the capacity of Gram-negative bacteria to develop new mechanisms of antibiotic resistance in the face of the introduction of new antimicrobial agents[8].

The high rate of ESBL producers in developing countries is clearly worrying; Lack of funds for effective infection control and limited access to effective antimicrobials have obvious implications for reducing morbidity and mortality associated with these infections. There is generally a lack of comprehensive data on extensive beta-lactamase-producing Enterobacteriaceae in African countries. However, there is sufficient evidence to highlight the widespread prevalence of beta-lactamases in Africa[9-13].

It is therefore necessary to put in place effective infection control practices to contain epidemics; and intervention strategies to reduce the selection and spread of these increasingly resistant pathogens. This is the motivation for this study which set itself the objectives of correlating the resistance profiles in Enterobacteriaceae to beta-lactams and quinolones because the presence of extended beta-lactamases is frequently associated with resistance to fluoroquinolones[14] by phenotypic methods and molecular identification of 14 genes encoding beta-lactamase to determine the level of extended beta-lactamases in enterobacteria isolated from community urinary infections which can cause different challenges. Indeed, there remains no doubt that bacteria producing extended beta-lactamases are of serious concern to the medical world. They are associated with increased morbidity and mortality and are often difficult and time-consuming to identify. When we add up the global prevalence rate which is increasing, and that of non-hospital settings, as well as the glaring lack of effective antimicrobial therapy, the future seems extremely worrying.

2. Methods

This is a cross-sectional study in a hospital environment carried out in the Bacteriology laboratories of the city of Lubumbashi for a period ranging from January 1, 2020 to October 21, 2022.

Urine samples were collected comprehensively: approximately 10 ml of freshly emptied mid-stream urine samples were collected into sterile, wide-mouthed, leak-proof containers. A well-mixed 10 μ l (0.01 ml) urine sample was inoculated using a calibrated metal loop into cysteine lactose electrolyte deficient (CLED) medium and then incubated at 37°C for 24 hours. under aerobic conditions. Samples showing significant bacteriuria ($\geq 10^5$ CFU/ml) were processed further, while samples producing $<10^5$ CFU/ml were considered insignificant.

Identification of bacterial isolates

Each culture plate was examined for growth of Enterobacteriaceae. Lactose fermenters and non-lactose fermenters were characterized by the oxidase test in order to distinguish enterobacteria that were always oxidase negative from non-enterobacteria that were oxidase positive. Finally, pure colonies were collected for identification on BIS.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. Bacterial suspensions were prepared by suspending the freshly cultured bacteria in 3 to 5 ml of normal saline and the turbidity was adjusted to 0.5 McFarland standard. A sterile cotton swab was dipped and rotated several times and pressed against the wall of the test tube. It was then applied to the entire surface of the Mueller Hinton agar. Antimicrobial susceptibility testing for all enterobacteria was performed using the disk diffusion method against ampicillin, amoxicillin, cefadroxil, cefuroxime, cefotaxime are used as first, second, third generation cephalosporin.[15]. Cefpodoxime susceptibility testing can lead to a high number of false-positive results which may be due to mechanisms other than extensive beta-lactamase production [16].

ESBL screening

Phenotypic detection which evaluates the capacity of the enzyme to hydrolyze certain cephalosporins and the capacity of clavulanic acid to counteract this hydrolysis. It is based on the choice of beta-lactams tested, their arrangement in the classic antibiogram by disk diffusion, the observation of the diameters of the inhibition zone, the synergies or antagonisms between certain antibiotics make it possible to describe phenotypes and to deduce the precise resistance mechanisms. Based on Ambler's structural or Bush's functional classifications[14]

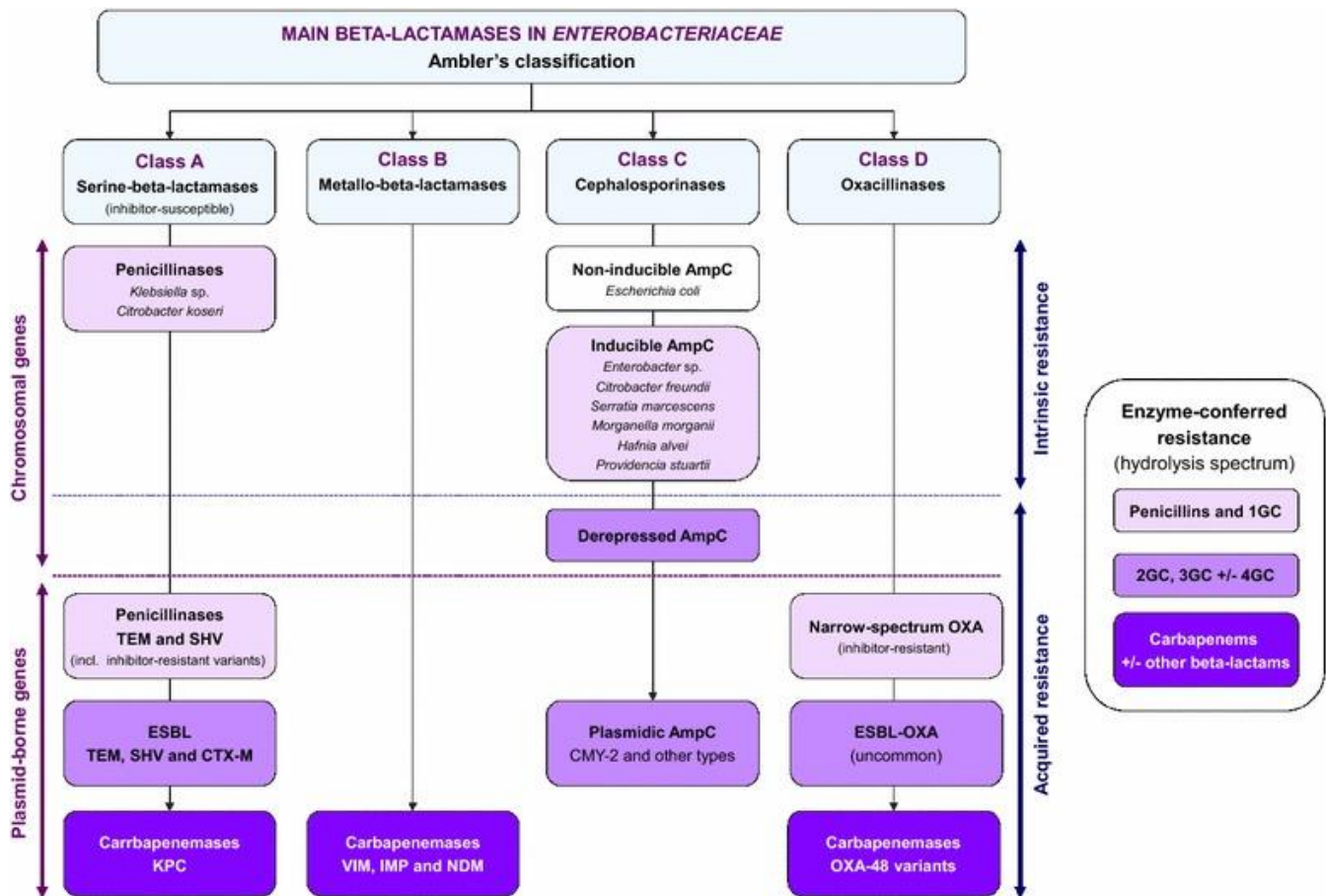


Fig. 1: “Intrinsic and acquired beta-lactamases in Enterobacteriaceae”. [17]

Phenotypic confirmation of ESBLs

β LSEs are inhibited *in vitro* by clavulanic acid. This inhibition by clavulanic acid is manifested on the antibiogram by the presence of images of synergy (called “champagne cork”) between the C3G and clavulanic acid (e.g.: AMC) distant 30 mm from the discs of cephalosporin.

In order to inhibit the effects of hyperproduction of cephalosporinase, the detection of the synergy image can be facilitated by bringing the cephalosporin discs closer to that of the disc containing clavulanic acid after a standard antibiogram on Mueller-Hinton agar added with 250 mg/L of cloxacillin (cephalosporinase inhibitor) this makes it possible to verify: that the resistance observed is indeed linked to this type of mechanism (restoration of sensitivity to the aforementioned molecules when there is no other mechanism of resistance to β -lactams) and to detect a possible associated extended-spectrum β -lactamase (ESBL) which would be masked by the hyperproduction of a cephalosporinase. [18]

Data and laboratory quality control

Standard Operating Procedures (SOPs) were strictly followed to verify that the media met the expiry date and quality control parameters. Culture media were prepared according to the manufacturer's instructions and sterility testing was performed before use. Visual inspections for gel cracks, bubbles and contaminations in the media were checked before use .

A quality control strain was used to check the quality of the medium. Each new batch was verified before use by testing *E. coli* ATCC 25922 growth control strains. When detecting ESBLs, ESBL-positive *K. pneumoniae* ATCC 700603 control strains and negative *E. coli* ATCC 25922 control strains for ESBLs were used. The clinical sample was collected according to the SOPs and immediately taken to the microbiology laboratory for bacteriological analysis. The culture results were carefully recorded before data entry and the data were double-checked before analysis.

Finally, we also tested the *Escherichia coli* strains with quinolones to correlate their induced resistance to the effects of extended spectrum beta lactamase .

Data analysis

Data was entered using Excel 365 software and exported to SPSS 23 for analysis.

3. Results and discussion

Escherichia coli was the most isolated enterobacterium from urine samples in the various laboratories in Lubumbashi, followed by *Citrobacter diversus* and *Enterobacter cloacae*. this observation is also that of several authors who also noted in their cohort that *Escherichia coli* was one of the most common isolates[19-22]. *Citrobacter spp* and *Enterobacter spp* were the bacteria isolated in situations of high resistance to antibiotics, particularly to cephalosporins, as noted by Sader HS et al [23], Gajdács M et al [24].

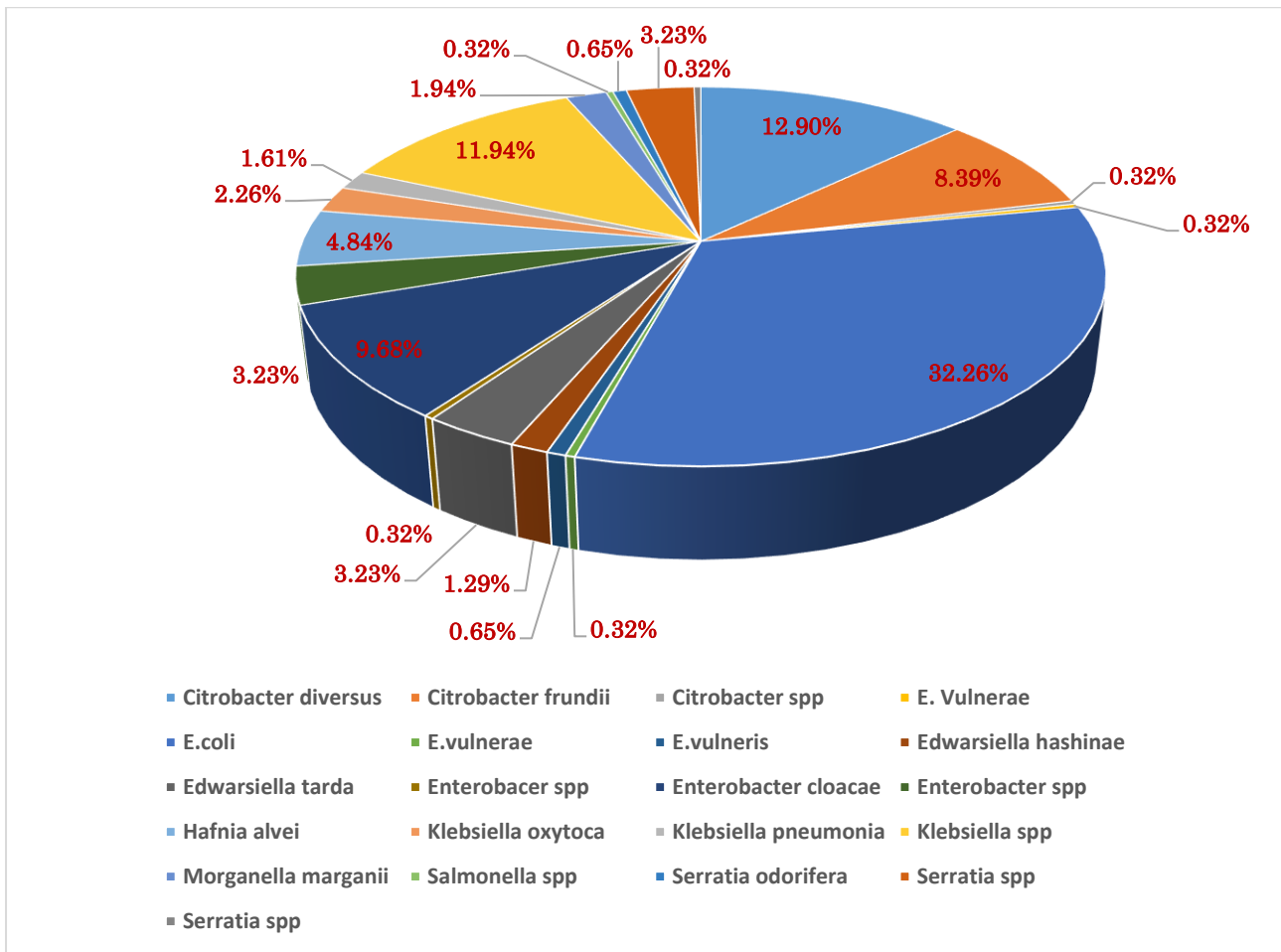


Figure 2. The frequency of Enterobacteriaceae strains

Table I. Resistance rates of beta-lactam enterobacteria strains

Germ	Ampicillin			Cefadroxil			Cefuroxime			cefotaxime			Amoxicillin			Amoxicillin + Clavulanic Ac		
	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S
Citrobacter diversus	0	32	2	2	11	19	7	17	10	5	20	9	0	29	3	7	20	7
Citrobacter freundii	0	22	2	5	9	10	5	12	7	9	10	5	3	16	5	10	4	10
Citrobacter spp	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1
E. Vulneris	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1
E.coli	6	80	5	15	45	31	23	33	35	35	30	26	14	67	10	39	25	27
E. vulneris	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1
E. vulneris	0	2	0	0	1	1	0	1	1	0	2	0	0	2	0	1	1	0
Edwarsiella hashinae	0	4	0	0	2	2	1	2	1	2	2	0	0	4	0	1	2	1
Edwarsiella delayed	0	9	0	1	5	3	1	7	1	1	8	1	1	7	1	1	8	1
Enterobacter spp	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0
Enterobacter cloacae	0	24	0	4	14	6	4	14	6	3	16	6	3	15	6	3	14	7
Enterobacter spp	1	6	0	1	4	2	2	4	1	2	4	1	2	4	1	1	6	0
Hafnia alvei	0	11	1	4	5	3	3	3	3	3	3	3	1	8	3	2	5	5
Klebsiella oxytoca	0	6	0	0	6	0	2	3	1	1	4	1	1	5	0	0	0	0
Klebsiella pneumonia	0	4	0	1	2	1	1	3	0	1	2	1	1	3	0	1	3	0
Klebsiella spp	0	29	2	5	15	9	6	11	14	5	9	17	2	20	9	2	10	19
Morganella morganii	0	6	0	2	3	1	3	2	1	2	3	1	2	3	1	3	2	2
Salmonella spp.	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0
Serratia odorifera	0	2	0	0	1	1	0	1	1	0	1	0	0	2	0	0	2	0
Serratia spp	1	9	0	1	1	8	2	4	4	2	7	1	2	6	2	0	5	5
Total	8	251	12	42	128	97	61	121	86	72	125	72	32	196	41	72	108	87
Percentage	92.61			47.94			45.14			46.46			72.86			40.44		

It appears from this table that the strains of isolated enterobacteria presented high rates of resistance, i.e. 92.61% for Ampicillin, 47.94% for cefadroxil, 45.14% for cefuroxime and 46.46% for cefotaxime, 72.86% for amoxicillin and 40.44% for amoxicillin + clavulanic acid.

At this level of the present study, the rates of Beta-lactam resistance were very high, much more for penicillins than for cephalosporins. Added to this is resistance to amoxicillin + clavulanic acid (inhibitor) estimated at 40.44%. In Brazil, Andressa Liberal Santos et al in their study on antimicrobial resistance in enterobacteria characterized a phenotypic profile of resistance to beta-lactam antibiotics among the 70 bacterial samples studied, 52 (74.28%) were resistant to ampicillin, 44 (62.85%) were resistant to amoxicillin combined with beta -clavulunate, lactamase inhibitor, 38 (54.28%) were resistant to cefazolin and 6 (8.57%) were resistant to cefuroxime [25] and In Burkina-Faso, The frequency of ESBL-PE was 58% (179 strains among the 308 Enterobacteriaceae isolates identified in the collected samples; 45% in outpatients and 70% in hospitalized patients)[26] and 49.3%). was found among clinical isolates from Ghana[27]

Enterobacteriaceae resistance to antibiotics, particularly of the β lactam type, is increasingly dominated by the mobilization of unique, continuously expressed genes that encode effective drug-modifying enzymes. Strong and pervasive selection pressure was apparently accompanied by a shift from "natural" resistance, such as inducible chromosomal enzymes, membrane impermeability characterized by violation of outer membrane permeability, and to modification from penicillin-binding target proteins and drug efflux to the modern paradigm of mobile gene pools that largely determine the epidemiology of modern antibiotic resistance[28,29].

Table II. Screening for extended beta-lactamase-producing Enterobacteriaceae strains

Germ	ATB on MH + 250mg/L cloxacillin (cephalosporinase inhibitor)	Synergy in champagne cork		
		Presence	Absence	Total
Citrobacter diverssus	3	4(20%)	13	20
Citrobacter freundii	1	4(40%)	5	10
Citrobacter spp	0	0	1	1
E. Vulneris	0	0	1	1
E.coli	8	9(30%)	13	30
E. vulneris	0	0	1	1
E. vulneris	0	0	2	2
Edwarsiella hashinae	0	1	1	2
Edwarsiella delayed	1	2	5	8
Enterobacer spp	0	1	0	1
Enterobacter cloacae	4	2(12.5%)	10	16
Enterobacter spp	1	1	2	4
Hafnia alvei	1	0	2	3
Klebsiella oxytoca	1	2	1	4
Klebsiella pneumonia	0	0	2	2
Klebsiella spp	1	3(33.33%)	5	9
Morganella marganii	0	1	2	3
Salmonella spp.	0	0	0	0
Serratia odorifera	1	0	0	1
Serratia spp	2	1(14.28%)	4	7
Total	24	31	70	125
Percentage	19.2%	24.8%	56%	100

The presence of extended spectrum Beta lactamase is 23.8% for all enterobacteria isolated from community urinary infections. Its rate is 30% in Escherichia coli, 20% in Citrobacter diverssus, 12.5% in Enterobacter cloacae and 14.28% in Serratia spp. We also note a hyperproduction of Cephalosporinase at 19.2% for all enterobacteria. This observation is not new because the WHO already stated that the proportion of ESBL-producing bacteria causing common infections in all regions of the world is high, which makes antibiotic resistance due to ESBLs a public health problem. major world[30]. The overall ESBL proportion estimate for East African hospitals (42%) is close to the estimates for Ghana (49%), Cameroon (54%), Gabon (45%) and Morocco (43%) [31]. Among enterobacteria, Moirongo observed

that the highest level of extended beta-lactamase was among *K. pneumoniae* (63.6%; 14/22) and *E. coli* (31.9%; 23/72), and the lowest among *S. enterica* (3.8%; 3/79).[32]

Table III. Resistance rate of *Escherichia coli* strains to quinolones

Germ	Ciprofloxacin			Levofloxacin			Norfloxacin			Nalidixic Acid		
	I	R	S	I	R	S	I	R	S	I	R	S
<i>E.coli</i>	5	60	26	3	67	21	3	45	43	5	76	10
Percentage	65.93			73.62			49.45			83.51		

The quinolone resistance rates in our cohort of uropathogenic *Escherichia coli* ranged from 83.51% to nalidixic acid, 73.62% to levofloxacin, 65.93% to ciprofloxacin and 49.45% to Norfloxacin and this situation is very worrying. Indeed, the WHO recently highlighted fluoroquinolone resistance in *Escherichia coli* and related organisms as the main threat to public health[33]. Our results are similar to those of Shenagari et al (2017) showed resistance of 45.3% to norfloxacin, 48.9% to ofloxacin, 50.2% to ciprofloxacin and 61.9% to nalidixic acid in Northern Iran.[34] In Nigeria, *E. coli* from urinary tract infections in Port-Harcourt presented resistance rates to nalidixic acid estimated at 68%, 60% to ciprofloxacin [35].

It is known that β -lactams (mainly extended-spectrum cephalosporins and carbapenems) and fluoroquinolones are the main therapeutic choices to treat infections caused by Enterobacteriaceae and quinolone resistance is multifactorial and can arise from one or more 'a combination of target site genetic mutations, increased production of multidrug resistance (MDR) efflux pumps, modifying enzymes and/or target protection proteins.[36,37]. Some studies report that ESBL production was associated with high co-resistance to fluoroquinolone (93% for ciprofloxacin), aminoglycosides (76.36% for gentamicin) and trimethoprim/sulfamethoxazole (95.65%) [38,39]. Indeed, at *E. coli* carrying the *qnr B* gene and positive for ESBL and carbapenemase phenotypes, *qnr* are genes which confer resistance to nalidixic acid and reduced sensitivity to fluoroquinolones and there is a frequent association of genes coding for spectrum β -lactamases expanded (ESBL) and these genes[40]

Conclusion

In this study, we report data on the resistance rate of enterobacteria and their rate of ESBL production on urine samples whose culture allowed the identification of enterobacteria. We found that increasing resistance among the most common human pathogenic Enterobacteriaceae is alarming because of the burden it poses to patients and public health. ESBL-producing Enterobacteriaceae isolates not only transmit resistance to beta-lactam antibiotics, but also frequently exhibit cross-resistance to other classes of antimicrobials including quinolones. This situation poses a significant public health challenge and highlights the urgent need to improve sanitation and implement antibiotic stewardship in developing countries.

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