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

Abstract

Introduction : Urinary tract infections can affect all individuals, regardless of gender and age , 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 : To determine 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 a growing global public health concern. associated with high morbidity and mortality, increased healthcare costs, and reduced gross domestic product (GDP)[1].In clinical setting beta-lactams are preferred over other antibiotics as they are considered effective and safe for use , due to their highly selective toxicity [2]. Beta lactams block the synthesis of peptidoglycan (or mucopeptide, or murein), which is the major polymer specific to the wall of Gram-negative and Gram-positive bacteria.[3-4] However , the growing beta-lactam resistance in Enterobacteriaceae and other Gram-negative organisms is mainly mediated by beta-lactamases [5], 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 [6] .

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[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].It's therefore necessary to, put in place effective control practices to contain epidemics and to reduce the selection and spread of these increasingly resistant pathogens.

However, the lack of information on the prevalence and antimicrobial profiles on beta lactamase producing bacteria makes it difficult to initiate infection control strategies. Furthermore, there is generally a lack of complete data on the numerous beta-lactamase-producing enterobacteria in African countries.[9-13]. , particularly in DR Congo where the sale of antibiotics in developing countries leaves much to be desired, we are witnessing a counter way in manufacturing and thus, Antimicrobial resistance takes a heavy toll on both patients and healthcare workers. In addition to the management made difficult by known treatment protocols which no longer respond, leading to deaths and disabilities, we note long periods of hospitalization and much more expensive treatments. It is in this context that we are carrying out this study which is focused on the search for Beta lactamase with extended spectrum in enterobacteria isolated from community infections.

Therefore ,the objectives of current study were to establish the prevalence and the antimicrobial resistance of beta lactamase producing bacteria isolated form patients' urines samples in Lubumbashi , DR Congo. This information will be used as a guideline for prevention and control of urinary tract infections as well as new research studies.

2. Methods

a. Stady area

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.

The city of Lubumbashi is in the south of the Democratic Republic of Congo. It is included in the square degree south 12127, in the square degree of the second parallel of the south of the equator and at the twenty-seventh meridian. Located at 11° 39 57 south latitude and 27° 28' 25" from the southern end of the upper Katanga sub-region although located in the heart of Africa,

b. Sample collection

A total of 10,234 urine samples were collected, including 1,219 from hospitalized patients and of which 271 gave rise to the isolation of enterobacteria and constituted the sample for the present study. Approximately 10 ml of freshly emptied mid-stream urine samples were collected into sterile, wide-mouthed, leak-proof containers. A well-mixed 10 µl (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.[14]

Identification of bacterial isolates

Each culture plate was examined for growth of Enterobacteriaceae. Suspected Enterobacteriaceae colonies were subjected to Gram stain, Catalase and Oxidase test to distinguish from other families. Finally, pure colonies were collected for identification on API 20 E.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for all enterobacteria against ampicillin, amoxicillin, cefadroxil, cefuroxim, cefotaxim, and amoxicillin + clavulanic acid using the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. In brief, 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 and applied to the entire surface of the Mueller Hinton agar. [15,16]

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]

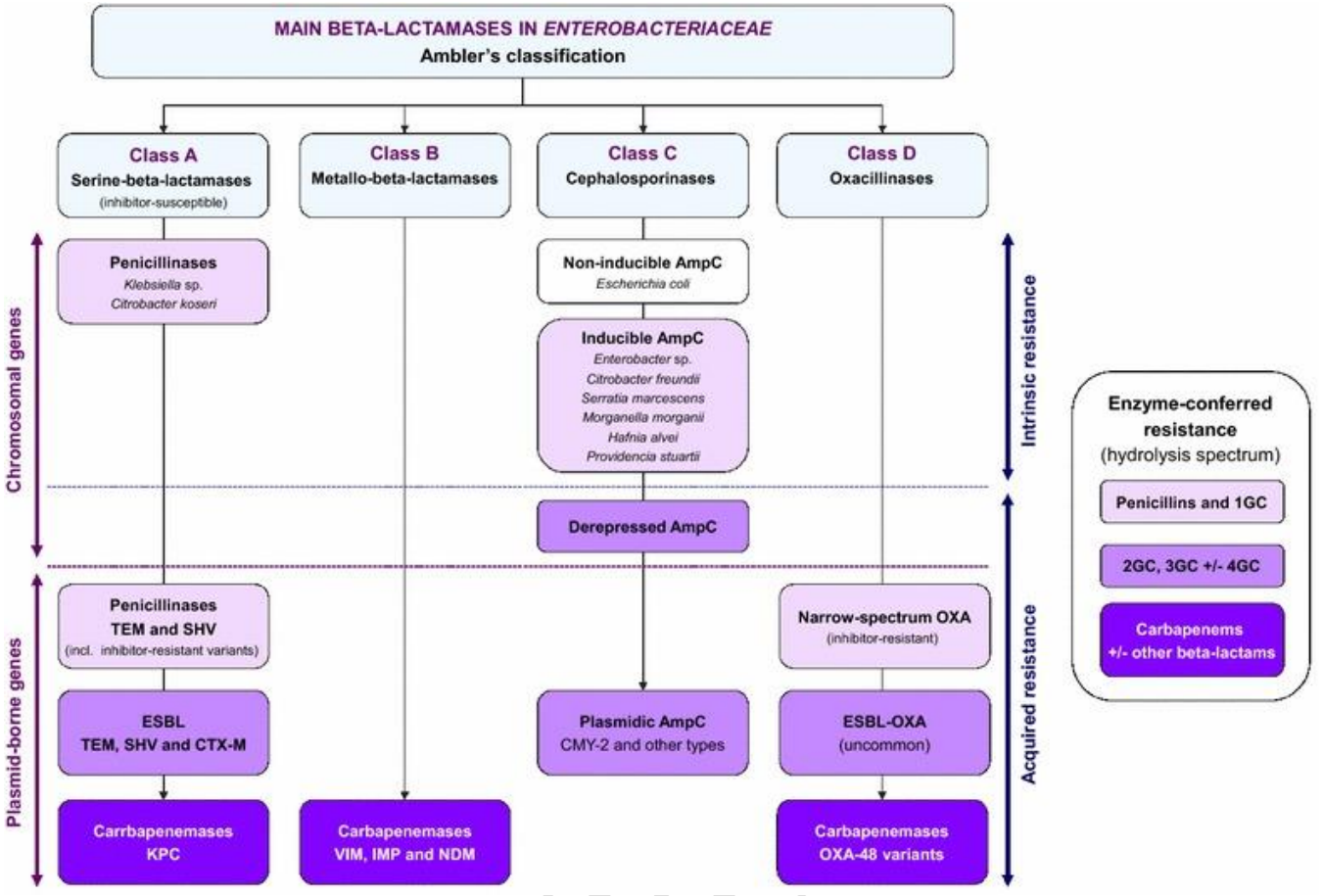

Fig. 1: Intrinsic and acquired beta-lactamases in Enterobacteriaceae.[17]

Phenotypic confirmation of ESBLs

βLSEs 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].

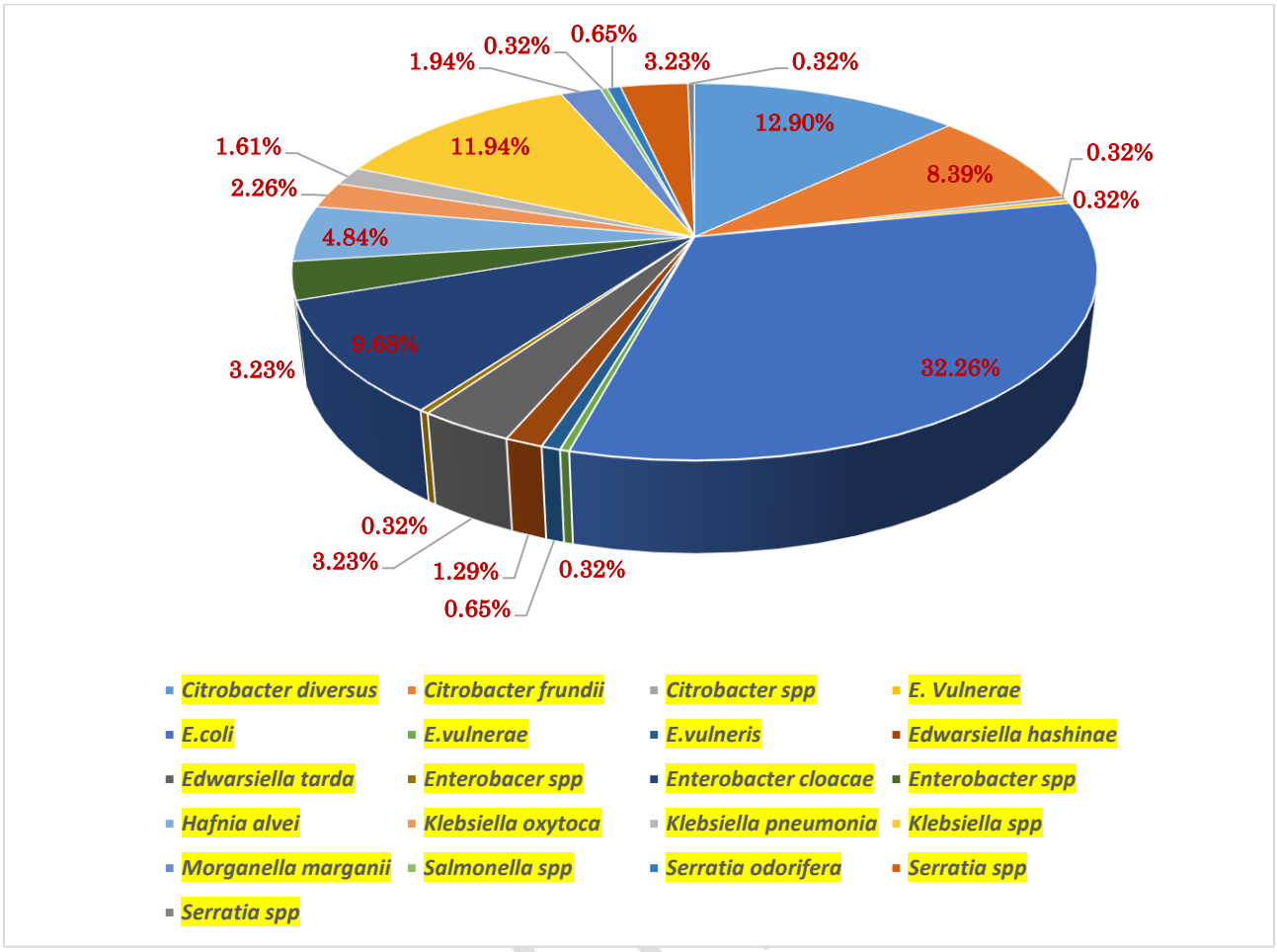

Figure 2. The frequency of Enterobacteriaceae strains

Table 1. Resistance rates of beta-lactam enterobacteria strains

Germ	Ampicillin (10µg)			Cefadroxil (30µg)			Cefuroxim (30µg)			Cefotaxim (30µg)			Amoxicillin (10µg)			Amoxicillin + Clavulanic Acid (300µg:20µg+10µg)		
	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S
Citrobacter diverssus	0	32	2	2	11	19	7	17	10	5	20	9	0	29	3	7	20	7
Citrobacter frundii	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
Enterobacer 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 marganii	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.

The rates of Beta-lactam resistance were very high, compared to that of cephalosporins. In addition, resistance to amoxicillin + clavulanic acid (inhibitor) was estimated at 40.44%. In Brazil, Andressa Liberal Santos et al in their study on antimicrobial resistance in enterobacteria and found that 52 (74.28%), 44(62.85%),38(54.28%) and 6(8.57%) were resistant to ampicillin, amoxicillin combined with beta -clavulanate, lactamase inhibitor, cefuroxime , respectively[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 2. 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 frundii	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 was 24.8% for all enterobacteria isolated from community urinary infections. Its rate among the most isolated bacteria is 30% in *Escherichia coli*, 20% in *Citrobacter diversus*, 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.[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].

Table 3. Resistance rate of Escherichia coli strains to quinolones

Germ	Ciprofloxacin (5µg)			Levofloxacin (5µg)			Norfloxacin (10µg)			Nalidixic Acid (30µg)		
	I	R	S	I	R	S	I	R	S	I	R	S
E.coli	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 *Escherchia 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[32]. 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.[33] In Nigeria, *Echerichia coli* from urinary tract infections in Port-Harcourt presented resistance rates to nalidixic acid estimated at 68%, 60% to ciprofloxacin [34].

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. 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.[35,36]. 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%) [37.38]. 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 b-lactamases expanded (ESBL) and these genes[39]

Conclusion

In this study, we report data on the resistance rate of enterobacteria and their rate of ESBL production on urine samples. 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.

References

1. O'Neill J.The review on antimicrobial resistance.2015http://amr-revieworg/sites/default/files/Report-5215pdf
2. Chant C., Leung A., Friedrich JO Optimal dosing of antibiotics in critically ill patients by using continuous/extended infusions: A systematic review and metaanalysis. Crit. Care. 2013;17:R279. doi:10.1186/cc13134. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
3. D. Cavallo, R. Fabre, F. Jehl, C. Rapp, E. Garrabé,Bêtalactamines,EMC - Maladies Infectieuses ;Volume 1, Issue 3,2004,Pages 129-202,ISSN 1638-623X,https://doi.org/10.1016/j.emcmi.2004.03.003.
4. https://microbiologie-clinique.com/beta-lactamine.html. 2023
5. (https://www.sciencedirect.com/science/article/pii/S1638623X04000125)
6. Rawat D, Nair D. Extended-spectrum β -lactamases in Gram Negative Bacteria. J Glob Infect Dis. 2010 Sep;2(3):263-74. doi:10.4103/0974-777X.68531. PMID: 20927289; PMCID: PMC2946684.
7. Emilia Hadziyannis, Marion Tuohy, Linda Thomas, Gary W. Procop, John A. Washington, Geraldine S. Hall,Screening and confirmatory testing for extended spectrum β -lactamases (ESBL) in *Escherichia coli, Klebsiella pneumoniae*, and *Klebsiella oxytoca* clinical isolates ☆ ,Diagnostic Microbiology and Infectious Disease,Volume 36, Issue 2,2000,Pages 113-117,ISSN 0732-8893,https://doi.org/10.1016/S0732-8893(99)00117-0.
8. Clinical and Laboratory Standards Institute (CSLI). Performance Standards for Antimicrobial Susceptibility Testing.30 Th ed. CSLI supplement M100(ISBN 978-1-68440-066-9)[Print];ISBN 978-1-68440-067-6[Electronuic]. Clinical and Laboratory Standards Institute, 950 West Vallery Road, Suite 2500, Wayne, Pennslyvania 19087 USA, 2020
9. Dhillon RH, Clark J. ESBLs: A Clear and Present Danger? Crit Care Res Pract. 2012;2012:625170. doi:10.1155/2012/625170. Epub 2011 Jun 6. PMID: 21766013; PMCID: PMC3135063.
10. Borg MA, Scicluna E, de Kraker M, et al. Antibiotic resistance in the southeastern Mediterranean—preliminary results from the ARMed project. *Euro Surveillance.* 2006;11(7):164–167. [PubMed] [Google Scholar]
11. Bouchillon SK, Johnson BM, Hoban DJ, et al. Determining incidence of extended spectrum *β* -lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centers from 17 countries: the PEARLS study 2001-2002. *International Journal of Antimicrobial Agents.* 2004;24(2):119–124. [PubMed] [Google Scholar]
12. Khalaf NG, Eletreby MM, Hanson ND. Characterization of CTX-M ESBLs in *Enterobacter cloacae, Escherichia coli* and *Klebsiella pneumoniae* clinical isolates from Cairo, Egypt. *BMC Infectious Diseases.* 2009;9, article 84 [PMC free article] [PubMed] [Google Scholar]
13. Kariuki S, Corkill JE, Revathi G, Musoke R, Hart CA. Molecular characterization of a novel plasmid-encoded cefotaximase (CTX-M-12) found in clinical *Klebsiella pneumoniae* isolates from Kenya. *Antimicrobial Agents and Chemotherapy.* 2001;45(7):2141–2143. [PMC free article] [PubMed] [Google Scholar]

14. Bayot ML, Bragg BN. Antimicrobial Susceptibility Testing. [Updated 2022 Oct 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539714/
15. https://www.cdc.gov/drugresistance/ar-lab-networks/domestic/expanded-ast.html
16. Wayne PA: Clinical and Laboratory Standards Institute. Wayne PA: Clinical and Laboratory Standards Institute; 2009. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement. CLSI document M100-S19. [Google Scholar]
17. Livermoore DM, Paterson DL. Pocket guide to extended spectrum β-lactamases in resistance. New Delhi: Springer (India) Private Limited; 2006. [Google Scholar]
18. Prasada Rao CMM, Vennila T, Kosanam S, Ponsudha P, Suriyakrishnaan K, Alarfaj AA, Hirad AH, Sundaram SR, Surendhar PA, Selvam N. Assessment of Bacterial Isolates from the Urine Specimens of Urinary Tract Infected Patient. Biomed Res Int. 2022 Jul 27;2022:4088187. doi:10.1155/2022/4088187. PMID: 35937407; PMCID: PMC9348933.
19. Vranic SM, Zatric N, Rebic V, Aljicevic M, Abdulzaimovic A. The Most Frequent Isolates from Outpatients with Urinary Tract Infection. Mater Sociomed. 2017 Mar;29(1):17-20. doi: 10.5455/msm.2017.29.17-20. PMID: 28484348; PMCID: PMC5402357.
20. Sharma D, Preston SE, Hage R (September 25, 2019) Emerging Antibiotic Resistance to Bacterial Isolates from Human Urinary Tract Infections in Grenada. Cureus 11(9): e5752. doi:10.7759/cureus.5752
21. Teferi, S., Sahlemariam, Z., Mekonnen, M. et al. Uropathogenic bacterial profile and antibiotic susceptibility pattern of isolates among gynecological cases admitted to Jimma Medical Center, Southwest Ethiopia. SciRep 13, 7078 (2023). https://doi.org/10.1038/s41598-023-34048-4
22. Thangavelu S, Dhandapani R, Arulprakasam A, Paramasivam R, Chinnathambi A, Ali Alharbi S, Durairaj K, Shrestha A. Isolation, Identification, Characterization, and Plasmid Profile of Urinary Tract Infectious Escherichia coli from Clinical Samples. Evid Based Complement Alternat Med. 2022 Mar 20;2022:7234586. doi:10.1155/2022/7234586. PMID: 35356239; PMCID: PMC8958076.
23. Sader HS, Mendes RE, Doyle TB, Davis AP, Castanheira M. Characterization of Enterobacter cloacae and Citrobacter freundii species complex isolates with decreased susceptibility to cephalosporins from United States hospitals and activity of ceftazidime/avibactam and comparator agents. JAC Antimicrob Resist. 2021 Aug 20;3(3):dlab136. doi:10.1093/jacamr/dlab136. PMID: 34430873; PMCID: PMC8378278.
24. Gajdács M, Urbán E. Resistance Trends and Epidemiology of Citrobacter-Enterobacter-Serratia in Urinary Tract Infections of Inpatients and Outpatients (RECESUTI): A 10-Year Survey. Medicine (Kaunas). 2019 Jun 18;55(6):285. doi:10.3390/medicina55060285. PMID: 31216725; PMCID: PMC6630883.
25. Santos AL, Dos Santos AP, Ito CRM, Queiroz PHP, de Almeida JA, de Carvalho Júnior MAB, de Oliveira CZ, Avelino MAG, Wastowski IJ, Gomes GPLA, Souza ACSE, Vasconcelos LSNOL, Santos MO, da Silva CA, Carneiro LC . Profile of Enterobacteria Resistant to Beta- Lactams. Antibiotics (Basel). 2020 Jul 15;9(7):410. doi: 10.3390/antibiotics9070410. PMID: 32679663; PMCID: PMC7400480.

26. Ouedraogo, AS., Sanou, M., Kissou, A. et al. High prevalence of extended-spectrum ß-lactamase producing enterobacteriaceae among clinical isolates in Burkina Faso. BMC Infect Dis 16, 326 (2016). https://doi.org/10.1186/s12879-016-1655-3
27. Obeng-Nkrumah N, Twum-Danso K, Krogfelt KA, Newman MJ. High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. Am J Trop Med Hyg. 2013;89(5):960–4.Return to ref 7 in articlePubMed Article
28. Iredell J, Brown J, Tagg K. Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. BMJ. 2016 Feb 8;352:h6420. doi:10.1136/bmj.h6420. PMID: 26858245.
29. Kotsyuba, KR, Voronkova, OS, VinnikovA. I., & Shevchenko, TM (2014). Mechanisms of antibiotic resistance of Enterobacteriaceae family representatives. Regulatory Mechanisms in Biosystems, 5(1), 33-38. https://doi.org/10.15421/021407
30. World Health Organization. ANTIMICROBIAL RESISTANCE: global report on surveillance. 2014.Return to ref 10 in article Google Scholar
31. Sonda, T., Kumburu, H., van Zwetselaar, M. et al. Meta-analysis of proportion estimates of Extended-Spectrum-Beta-Lactamase-producing Enterobacteriaceae in East Africa hospitals. Antimicrob Resist Infect Control 5, 18 (2016). https://doi.org/10.1186/s13756-016-0117-4
32. WHO (2014). Antimicrobial Resistance: Global Report on Surveillance 2014. Genova. [Google Scholar]
33. Pouladfar G, Basiratnia M, Anvarinejad M, Abbasi P, Amirmoezi F, Zare S. The antibiotic susceptibility patterns of uropathogens among children with urinary tract infection in Shiraz. Medicine (Baltimore). 2017;96(37):e7834. doi: 10.1097/MD.0000000000007834 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
34. Adebola Onanuga et al. Phenotypic and molecular characterization of antimicrobial resistant Escherichia coli from urinary tract infections in Port-Harcourt, Nigeria. Pan African Medical Journal. 2019;34:144. [doi: 10.11604/pamj.2019.34.144.18182]
35. Liam S. Redgrave, Sam B. Sutton, Mark A. Webber, Laura JV Piddock, Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success,Trends in Microbiology,Volume 22, Issue 8,2014,Pages 438-445 ,ISSN 0966-842X,https://doi.org/10.1016/j.tim.2014.04.007.
36. Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. Ann NY Acad Sci. 2015 Sep;1354(1):12-31. doi:10.1111/nyas.12830. Epub 2015 Jul 17. PMID: 26190223; PMCID: PMC4626314.

37. Diagbouga S, Salah FD, Sadji AY, Metuor Dabire A, Nadembega C, Banla Kere A, et al. Detection of high prevalence of TEM/SHV/CTX-M genes in ESBL producing and multidrug resistant *Klebsiella Pneumoniae* and *Klebsiella Oxytoca*. J Clin Diagn Res. 2016;4(1:130):p7.Return to ref 15 in article

38. Salah FD, Diagbouga S, Metuor Dabire A, Sadji AY, Nadembega C, Moumouni A, et al. First detection of Resistance genes encoding extended Spectrum β-lactamase producing *Escherichia coli* at Lomé, Togo. Arch Clin Microbiol. 2017;7(6):p7.

39. Dembélé R, Konaté A, Traoré O, Kaboré WAD, Soulama I, Kagambèga A, Traoré AS, Guessennd NK, Aidara-Kane A, Gassama-Sow A, Barro N. Extended spectrum beta-lactamase and fluoroquinolone resistance genes among *Escherichia coli* and *Salmonella* isolates from children with diarrhea, Burkina Faso. BMC Pediatr. 2020 Oct 3;20(1):459. doi:10.1186/s12887-020-02342-z. PMID: 33010801; PMCID: PMC7532561.