



Antimicrobial Resistance Profile and Molecular Characterization of Extended-spectrum Beta-lactamase Genes in Enterobacteria Isolated from Human, Animal and Environment

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GMVC, GNK and OMB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GMVC, OMB, OGD and TKB managed the analyses of the study. Authors AA, TAA, KKI, DSK, TE, Konate Ali and KF managed the literature searches, contributed to the writing and editing of the manuscript. Authors Kamenan Alphonse and DM supervised all the laboratory activities and the final manuscript submitted. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The aim of this study is to determine the antibiotic resistance profile and characterize extended spectrum beta-lactamase gene of enterobacteria strains isolated from human biological products, fecal matter of animals and the environment.

Materials and Methods: Enterobacteria producing ESBL strains were isolated from human products, fecal matter of healthy animals (cattle, sheep and pigs) intended for human consumption and environment (hospital effluents and municipal sewage) using homemade medium (Drigalski supplemented with 2 mg/L of ceftazidime). Resistance to beta-lactams has been evaluated by the diffusion method was carried out as recommended by NCCLS. Characterization of Beta-Lactamase resistance genes (*blaCTXM*, *blaSHV*, *blaTEM*, *blaGES*, *blaPER* and *blaVEB*) was performed by simplex and multiplex PCR.

Results: The strains were resistant to antibiotics from beta-lactam family (penicillin with inhibitor, monobactam, cephalosporin) but no resistant was observed to carbapenem (imipénème, méropénème). All resistance genes were identified in environment strains.

Conclusion: This study showed the presence of common beta-lactam resistance genes (*blaTEM*, *blaSHV* and *blaCTX-M*) to human, animal and environment. The risk of dissemination and circulation of ESBL enterobacteria between animals, humans and the environment exists in Ivory Coast because of the absence of a barrier between them.

Keywords: *Enterobacteria ESBL; human; animal; environment; Ivory Coast.*

1. INTRODUCTION

Antibiotics are widely used not only to treat human and animal infections but also in farms and aquacultures, as food additives to promote animal growth and prevent diseases. Unfortunately, the intensive use and misuse of antibiotics in these different domains have resulted in antibiotic resistance among bacteria such as enterobacteria producing extended-spectrum β -lactamase [1,2]. Antibiotic resistance is not confined to bacteria involved in clinical infections alone but also present in bacteria in the aquatic environment and animal production, which might be an important contributory factor in the spread of resistance [3,4]. There are two main mechanisms involved in the development of antibiotic resistance, namely mutation [5] and acquisition of resistance genes [6] by horizontal gene transfer (HGT).

The most abundant ESBL types are represented by SHV, TEM and CTX-M. However, a variety of other enzymes such as VEB, GES/IBC, PER, BEL, and oxacillinases with ESBL activity have been described worldwide [7]. Plasmid-encoded Extended-spectrum β -lactamases (ESBLs) are increasingly spreading among Enterobacteriaceae from human, animal and environment isolates throughout the world due mostly to their presence on highly conjugative plasmid [8]. ESBLs have the ability to inactivate by hydrolyse most β -lactam antibiotics, including oxyimino- β -lactams such as

ceftazidime, ceftiofur, and aztreonam. They do not hydrolyze cephamycins and carbapenems and they are inhibited by clavulanic acid [10].

Originally, ESBLs were mainly demonstrated in hospital environment but now high frequencies of antimicrobial resistance have been found in enterobacteria, in fecal flora as well as in clinical isolates [9]. Also, the release of antibiotics in large amounts into natural ecosystems through hospital and municipal waste waters untreated can impact the structure and activity of environmental microbial populations. Acquired resistance to β -lactam antibiotics in gram-negative bacteria is mainly mediated by bacterial β -lactamases and the emergence of extended-spectrum β -lactamases (ESBLs) is of great clinical importance. The increase of these bacteria and their spreading in the hospital has been well documented in the world [10,11] and in Ivory Coast [12,13]. Very patchy data on enterobacteria producing extended spectrum β -lactamases in the fecal flora of animal and environment (hospital effluents and municipal wastewater) in Ivory Coast have been published, and thus we know surprisingly little about the enterobacteria ESBL and their resistance genes outside clinical environment. It is important, therefore, to document the occurrence and types of antibiotic resistance genes in the environment and animal.

The aim of this study is to determine the antibiotic resistance profile and characterize

extended spectrum beta-lactamase genes of enterobacteria strains isolated from human biological products, fecal matter of animals and the environment.

2. MATERIALS AND METHODS

2.1 Sampling Collection

This study was carried out from December 2012 to November 2013 in Abidjan (Ivory Coast). Human enterobacteria strains producing extended spectrum beta-lactamases (ESBL) were obtained from the clinical bacteriology unit (CBU) of the Institut Pasteur of Ivory Coast. These strains were isolated from biological products (urine, blood and pus) of hospitalized and nonhospitalized patients. In the same period, ESBL enterobacteria strains were isolated from the fecal matter of healthy animals (cattle, sheep and pigs) intended for human consumption. In the environment, ESBL enterobacteria strains were isolated from hospital effluents and municipal sewage.

2.2 Isolation and Identification of ESBL Enterobacteria Strains

All ESBL producing enterobacteria strains were isolated on Drigalski supplemented with 2 mg/ml of ceftazidime [14] and were identified using the API 20E galerie (bioMérieux, Marcy l'Etoile, France).

2.3 Antibiotic Susceptibility Testing

The antimicrobial susceptibility of the extended spectrum enterobacteria β -lactamase isolates was determined by the Bauer-Kirby disk diffusion test using antibiotic disks (Bio-Rad, France) [15]. The double synergy test was used for detection of ESBL-producing strains. The disks of cefotaxime (30 μ g), ceftazidime (30 μ g), céfépime (30 μ g) and ceftriaxone (30 μ g) were placed around an amoxicillin/clavulanic acid disk (10/20 μ g) on Mueller Hinton agar (BioMérieux, France). The distance between the discs, center to center was 20 mm. This test was performed when the strain was categorized intermediate or resistant to third generation cephalosporins. Of these, sixteen antimicrobial agents from four antibiotic families (β -lactams, quinolones, aminosides and cyclins) were tested. Clinical Laboratory Standards Institute (CLSI) guidelines were followed for inoculum standardization, medium and incubation conditions, and internal quality control organisms (*E. coli* ATCC 25922). Isolates were screened for the ESBL-producing phenotype by the standard double-disc synergy test, as described previously [16]. Antimicrobial discs (concentration of antibacterial in μ g) used were amoxicillin/clavulanic acid (10/20), ceftazidime (30), ceftriaxone (30), cefotaxime (30), cefepime (30), ceftiofur (30), imipenem (10), meropenem (30), aztreonam (30), nalidixic acid (30), ciprofloxacin (5), amikacin (30), gentamycin (15), tetracycline (30),

Table 1. Primers used in the study

Genes <i>bla</i>	Primers	Sequence (5'->3')	Position	PCR product size (pb)	Accession number
<i>TEM</i>	a216 (+)	ATAAAATTCTTGAAGACGAAA	1-21	1079	AB282997
	a217 (-)	GACAGTTACCAATGCTTAATCA	1080-1059		
<i>SHV</i>	os-5 (+)	TTATCTCCCTGTTAGCCACC	23-42	795	X98098
	os-6 (-)	GATTTGCTGATTTGCTCGG	818-799		
<i>PER</i>	per (+)	CCTGACGATCTGGAACCTTT	465-485	716	721957
	per (-)	GCAACCTGCGCAAT(GA)ATAGC	1181-1161		
<i>VEB</i>	veb (+)	ATTTCCCGATGCAAAGCGT	351-370	542	AF010416
	veb (-)	TTATTCCGGAAGTCCCTGT	893-875		
<i>GES</i>	ges (+)	ATGCGCTTCATTACGCAC	1332-1350	863	AF156486
	ges (-)	CTATTTGTCCGTCTCAGGA	2195-2176		
<i>CTXM-1</i>	ctxM1(+)	GGTTAAAAATCACTGCGTC	65-84	863	X92506
	ctxM1(-)	TTGGTGACGATTTTAGCCGC	928-909		
<i>CTXM-2</i>	ctxM2(+)	ATGATGACTCAGAGCATTCTG	6-25	865	X92507
	ctxM2(-)	TGGGTACGATTTTCGCCGC	871-852		
<i>CTXM-8</i>	CtxM8(+)	GCGGCGCTGGAGAAAAGCAG	712-731	608	AF189721
	CtxM8(-)	GCTGCCGTTTTATCCCGA	6336-6355		
<i>CTXM-9</i>	ctxM9(+)	ATGGTGACAAAGAGAGTGCA	6336-6355	869	AF174129
	ctxM9(-)	CCCTTCGGCGATGATTCTC	7205-7187		

minocycline (30) and tigecycline (30). All the antibiotics were procured from Bio-rad (France). Only included in the study, were ESBL enterobacteria showing resistance to beta-lactamins, quinolones, aminoglycosides and cyclins.

2.4 PCR Amplification of Beta-lactamase Genes

Plasmid DNA was used for detection of β -lactamases and was extracted using Mini prep K0502 kit (Fermentas, Vilnius, Lithuania). The ESBL gene was characterized by polymerase chain reaction as described by [12]. PCR amplification was performed in a final reaction volume of 50 μ l. Primers used in this study are given in Table 1. The reaction mixture contained a PCR Reaction Buffer, 10x concentrated with 20 mM MgCl₂, PCR Grade Nucleotide Mix (2.5 mM each), specific primers for each target (20 pmol) and a FastStart Taq DNA Polymerase, 5 U/ μ l (Roche). The PCR conditions were carried out in a thermocycler UNOII (BIOMETRA®). Amplification products were analyzed by electrophoresis in a 2% agarose gel (Invitrogen) stained with ethidium bromide and visualized under Ultra Violet light.

The cycling conditions for amplification were as follows: for blaTEM, initial denaturation at 94°C for 1 min and 30 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C, followed by 7 min at 72°C; for blaSHV, PER, VEB, GES et CTXM gene, initial denaturation of 1 min at 94°C and 30

cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, followed by 7 min at 72°C.

3. RESULTS

3.1 Enterobacteria ESBL Strains

The human strains consisted of 70 species of ESBL dominated predominantly by the species *Escherichia coli* (37,1%), *Klebsiella pneumoniae* (30,6%) and *Enterobacter cloacae* (17,7%). The animal strains (239 species of ESBL) were distributed as follows: 81 strains from cattle, 60 from sheep, and 98 from pigs. These strains consist primarily of *Escherichia coli* strains with over 87,4%. Environment ESBL strains, 130 and 127 species were isolated respectively from hospital effluents and municipal wastewater. The main species were *Escherichia coli* (36,9%), *Klebsiella pneumoniae* (15,2%) and *Enterobacter aerogenes* (14,9%).

3.2 Enterobacteria ESBL Resistance Rates according to Their Origins

The average levels of resistance to third generation and fourth generation (CRO, CTX, CAZ, and FEP) cephalosporins for all strains of human, animal and environmental origin ranged from 97.2% to 100%. There was no significant difference ($P > 0.05$) exist between these levels regardless of the origin of the ESBL strains. Mean resistance levels for amoxicillin + clavulanic acid (AMC) were higher in strains of human origin (87.1%) followed by strains of environmental origin (64.5%). A significant

Table 2. Diversity of ESBL strain isolated from various origins

ESBL species	Origins		
	Human (%)	Animal (%)	Environment (%)
<i>Escherichia coli</i>	37,1	87,4	36,9
<i>Escherichia vulneris</i>	0,0	1,7	2,0
<i>Klebsiella pneumoniae</i>	30,6	2,0	15,2
<i>Klebsiella oxytoca</i>	3,2	0,0	9,7
<i>Enterobacter aerogenes</i>	1,6	3,7	14,9
<i>Enterobacter cloacae</i>	17,7	0,0	3,0
<i>Enterobacter amnigenes</i>	0,0	0,0	1,2
<i>Citrobacter freundii</i>	1,6	0,0	7,0
<i>Citrobacter koseri</i>	0,0	6,2	2,6
<i>Proteus mirabilis</i>	0,0	0,0	3,2
<i>Proteus vulgaris</i>	0,0	0,0	0,4
<i>Citrobacter amalonaticus</i>	0,0	0,0	0,4
<i>Serratia marcescens</i>	0,0	0,0	3,0
<i>Levinea sp</i>	1,7	0,0	1,5

Table 3. Enterobacteria ESBL resistance rates according to their origins

Antibiotics (load in µg)	Mean resistance (%) according to their origins		
	Human	Animal	Environment
AMC (20/10)	87,1 ^d	49,3 ^c	64,5 ^e
CRO (30)	100 ^a	100 ^a	100 ^a
CTX (30)	100 ^a	99,2 ^a	99,6 ^a
FEP (30)	100 ^a	100 ^a	97,2 ^a
CAZ (30)	100 ^a	100 ^a	100 ^a
ATM (30)	100 ^a	100 ^a	99,6 ^a
FOX (30)	36,1 ^c	3,6 ^b	24,6 ^c
IPM (10)	0 ^b	0 ^b	0 ^b
MEM (10)	0 ^b	0 ^b	0 ^b

AMC = amoxicillin + clavulanic acid; FEP = cefepime; FOX = ceftaxime; CAZ = ceftazidime; CTX = cefotaxime;

CRO = ceftriaxone; ATM = aztreonam; IPM = imipenem; MEM = meropenem

In line and column, the values assigned to the same letter are not significantly different at the 5% threshold according to the Newmann-Keuls test

Table 4. ESBL genes and their associations in the different strains

Beta-lactamases genes	Human (%) N=24	Animal (%) N=71	Environment (%) N=69
<i>bla</i> _{TEM}	7 (29,2)	18 (25,2)	9 (13)
<i>bla</i> _{SHV}	4 (16,7)	20 (28,2)	7 (10)
<i>bla</i> _{CTX-M1}	5 (20,8)	5 (7)	2 (2,9)
<i>bla</i> _{CTX-M2}	2 (8,3)	4 (5,6)	2 (2,9)
<i>bla</i> _{CTX-M8}	0 (0,0)	0 (0,0)	2 (2,9)
<i>bla</i> _{CTX-M9}	0 (0,0)	7 (9,9)	2 (2,9)
<i>bla</i> _{GES}	0 (0,0)	1 (1,4)	1 (1,4)
<i>bla</i> _{PER}	0 (0,0)	0 (0,0)	1 (1,4)
<i>bla</i> _{VEB}	0 (0,0)	0 (0,0)	0 (0,0)
Beta-lactamases genes associations			
<i>bla</i> _{TEM / SHV}	1 (4,2)	5 (7)	3 (4,3)
<i>bla</i> _{TEM / CTX-M}	2 (8,3)	5 (7)	13 (18,8)
<i>bla</i> _{SHV / CTX-M}	2 (8,3)	6 (8,5)	15 (21,7)
<i>bla</i> _{TEM / SHV / CTX-M}	1 (4,2)	0 (0,0)	1 (1,4)
<i>bla</i> _{TEM / SHV / VEB}	0 (0,0)	0 (0,0)	1 (1,4)
<i>bla</i> _{TEM / SHV / CTX-M / GES}	0 (0,0)	0 (0,0)	4 (5,8)

difference (P <0.05) was observed between the mean strain resistance of AMC strains. However, the mean levels of resistance to cephamycins were 36.1% and 24.6% for strains of environmental and human origin respectively. No resistance was observed for carbapenems (imipenem and meropenem).

3.3 Search Beta-lactamases (*bla*) Genes of ESBL Strains according to Their Origin

All resistance genes and their different associations were detected in environmental strains. In animal strains, however, only a few of these genes and their associations have been detected. No association of resistance genes was detected in human strains during the study.

4. DISCUSSION

This study presents the diversity antimicrobial resistance profile and betalactamase gene of Enterobacteria producing broad-spectrum.

BlaCTXM -1 and blaTEM genes were the most identified, followed by the blaSHV genes, whereas genes association such as blaTEM / blaSHV / blaCTX-M-1, blaTEM / blaCTX -M-1, blaSHV / blaCTX-M-1 and blaTEM / blaSHV were observed in low frequency. The proportions found are lower than those of Guessenn and al. [12] in human Enterobacteriaceae producing broad-spectrum beta-lactamases in Abidjan. Their proportions were 65.6%, 64.9% and 48.3% for blaCTX- M-1, blaTEM and blaSHV respectively. The results of this study are also

lower than those of Mohammad *and al.* [17] who reported high proportions of blaCTX-M-1 genes (46.5%), blaTEM (54%) and blaSHV (67.4%) in their study of *Klebsiella pneumoniae* strains in Teheran. They also demonstrated beta-lactamases genes associations with 23.2% and 19.2% for blaTEM / blaCTX-M and blaTEM / blaSHV / blaCTX-M respectively.

Several studies have shown an alarming increase of blaCTX-M of ESBLs with a strong predominance type [10,18]. In other studies in Tunisia on *Escherichia coli* ESBL strains isolated from children, Réjiba and al. [19] showed a high proportion of 97% blaCTX-M gene. The majority of the blaCTX-M genes belonged to group 1. The blaSHV had an occurrence of 6% while the blaTEM gene was not detected. High levels of blaCTX-M genes, low blaSHV and blaTEM gene frequency have also been reported in Algeria [20], Thailand [21], Switzerland [22] and in Saudi Arabia [23] in Enterobacteria BLSE strains isolated from clinical specimens.

The blaTEM, blaSHV and blaCTX-M genes of the ESBL family have also been identified in animal enterobacteria ESBL strains. Unlike to human strains with the blaCTX-M gene (blaCTX-M-1) was predominance, the genes blaSHV and blaTEM were most identified in animal strains. These two genes are responsible for resistance to beta-lactam of more than 50% of the Enterobacteria BLSE strains in cattle, sheep and pigs. However, a new group of blaCTX-M, blaCTX-M-9 has been identified in some animal's EBLSE strains. These results could be explained by a naturally high prevalence of certain plasmid types and subtypes harbored by *E. coli* isolated from animal, which in fact constituted a preferred host of ESBL genes. Indeed, according to Haenni and al. [24] (Plasmid Inc11 / ST3, for example) are often found in bacteria without epidemiological links and belonging to many animal species (dog, cat, cow, horse, goat, Hen, sheep).

In this study, distributions of the different types of ESBL genes identified in enterobacteria producing broad-spectrum beta-lactamases strains of animal origin corroborates distributions reported in Europe [25,26]. The blaTEM-52, blaSHV-12, and blaCTX-M-1 genes are the most frequently reported types in order of importance in non-human reservoirs such as live animals or in the processing chain of these animals [27].

However, Felix and al. [28] showed a predominance of the blaCTX-M-1 gene in

Escherichia coli strains isolated from carcass and caecum from healthy broiler chickens. Horton and al. [29] also reported the prevalence of *Escherichia coli* ESBL with blaCTX-M-1 gene in the stool of cattle, pigs and chickens in the UK with higher isolation rates than other animal species intended Consumption.

Furthermore, there is evidence that for *Salmonella* sp. and enteropathogenic *Escherichia coli*, producing ESBL enzyme and responsible for food infections, is an example of direct transmission of these genes from animals to humans [10,30].

As far as transmission to humans is concerned, the scientific evidence supports the existence of two distinct bacterial reservoirs, human and animal. However, some identical ESBL plasmids such as those carrying the blaCTX-M-15 gene have been described in humans and in cattle [31]. Comparison between human and animal strains established that these are more plasmids (Inc11 / ST3) than the bacterial populations that are found identical between humans and animals [32].

The problem of antibiotic resistance genes is not limited to hospital and animal strains, resistance is also present in bacteria of environmental (hospital effluents and domestic wastewater) origin. This work has shown that municipal wastewater and hospital effluents represent a source or reservoir of antibiotics resistant bacteria and antibiotic resistance genes that could be transmissible to humans. Thus, the aquatic environment could be an important factor in the spread of resistance as indicated by Zhang and al. [4]. In addition, contamination of wastewater with antibiotic residues can also lead to selective pressure on antibiotic-resistant bacteria and resistant genes that can pose a risk to human and even animal health [33].

In this study, all the genes found in EBLSE strains of human and animal were also identified in the environment in addition to new resistance genes, including blaGES, blaVEB and blaPER genes called "new ESBLs". Gene combinations were much higher in hospital effluent strains than in municipal effluents. The high proportion of association of resistance genes in hospital effluents could be explained by large sizes of plasmids harbored by these strains. The plasmids sizes can often reach up to 104 base pairs and would allow bacteria to survive in hostile environments (hospital and municipal effluents). Indeed, according to Reinthaler and al.

[34], the plasmids of ESBL enterobacteria in the environment and especially in hospital effluents are known to contain within them several plasmids harboring numerous antibiotic resistance genes. In addition, these plasmids are able to autotransfer from one bacteria to another and replicate independently in hosts [4]. All these facts make hospital effluents a reservoir of resistance genes.

Some authors have already noted the presence of a wide variety of resistance genes in enterobacteria producing broad-spectrum beta-lactamase, including *Escherichia coli*. These authors have demonstrated all resistance genes including the genes of new ESBLs that may be involved in antibiotic resistance [35,36].

5. CONCLUSION

The genotypic profile of isolated enterobacteria producing broad-spectrum beta-lactamases from human, animal and environment strains showed the presence of common beta-lactam resistance genes (*blaTEM*, *blaSHV* and *blaCTX-M*) and unusual resistance genes (*blaPER*, *blaVEB* and *blaGES*). Hospital effluents appear to be an important reservoir of strains with resistance genes. Some genes not detected in humans and animals, are present in these effluents. These hospital effluents discharged without treatment into surface water can be a source of dissemination of potentially pathogenic enterobacteria ESBL that can cause public health problems. The risk of dissemination and circulation of ESBL enterobacteria between animals, humans and the environment exists in Côte d'Ivoire because of the absence of a barrier between them. The dissemination and circulation of ESBL enterobacteria is a public health problem.

ETHICAL APPROVAL

As per international standard and university standard ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Mazel D, Davies J. Antibiotic resistance in microbes. *Cell Mol Life Sci*. 1999;56(9-10): 742-54.
- Hirsch R, Ternes T, Haberer K, Kratz KL. Occurrence of antibiotics in the aquatic environment. *Sci Total Environ*. 1999; 225(1-2):109-18.
- Bywater RJ. Identification and surveillance of antimicrobial resistance dissemination in animal production. *Poultry Science*. 2005;84(4):644-8.
- Zhang Y, Marrs CF, Simon C, Xi C. Wastewater treatment contributes to selective increase of antibiotic resistance among *Acinetobacter spp*. *Science Total Environment*. 2009;407(12):3702-3706.
- Martinez JL, Baquero F. Mutation frequencies and antibiotic resistance. *Antimicrob Agents Chemother*. 2000;44(7): 1771-7.
- Davies J. Inactivation of antibiotics and the dissemination of resistance genes. *Science*. 1994;264:375-382.
- Paterson DL, Bonomo RA. Extended-spectrum β -lactamases: A clinical update. *Clinical Microbiology Review*. 2005;18:657-86.
- Schwarz S, Chaslus-Dancla E. Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Veterinary Research*. 2001;32:201-25.
- Bonnet R. Growing group of extended-spectrum β -lactamases: The CTX-M enzymes. *Antimicrobial Agents and Chemotherapy*. 2004;48:1-14.
- Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Herman L. Broad-spectrum beta-lactamases among *Enterobacteriaceae* of animal origin: Molecular aspects, mobility and impact on public health. *FEMS Microbiol Review*. 2010;34:295-316.
- Castanheira M, Rodrigo E, Mendes, Ronald N, Jones, Helio S, Sader. Changes in the frequencies of β -lactamase genes among *Enterobacteriaceae* isolates in U.S. Hospitals, 2012 to 2014: Activity of ceftazidime-avibactam tested against β -lactamase-producing isolates. *Antimicrob Agents Chemother*. 2016;60(8):4770-4777.
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- Guessenn N, Kacou-N'douba A, Gbonon V, Yapi D, Ekaza E, Dosso M, Courvalin P. Prévalence et profil de résistance des entérobactéries productrices de bêta lactamase à spectre élargi à Abidjan de 2005 à 2006. *Journal of Science*

- Pharmaceutical and Biology. 2008a;9(1): 63-70.
13. Guessennd KN, Toty AA, Gbonon MC, Dondelinger M, Toé E, Ouattara MB, Tiékoura B, Konan F, Dadié AT, Dosso M, Galleni M. CTX-M-15 extended-spectrum- β -lactamase among clinical isolates of Enterobacteriaceae in Abidjan, Côte d'Ivoire. International Journal of Biology Research. 2017;2(3):05-08.
 14. Ouattara MB, Guessennd KN, Koffi-Nevry R, Koffi S, Ouattara GD, Gbonon V, Tiekoura KB, Faye-Kette H, Dosso M. Evaluation of Drigalski agar supplemented with ceftazidime (2 mg/L) for the isolation of spectrum betalactamase (ESBL) producing Enterobacteria. Afr. J. Microbiol. Res. 2014;8(89):2758-2765.
 15. Bauer AW, Kirby WMM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1966;45:493-496.
 16. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactams in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. Review Infectious Disease. 1988;10:867-878.
 17. Mohammad Mehdi Feizabadi, Somayeh Delfani, Nafiseh Raji, Araz Majnooni, Marzieh Aligholi, Fereshteh Shahcheraghi, Mahmood Parvin, Davud Yadegarinia. Distribution of blaTEM, blaSHV, blaCTX-M genes among clinical isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran. Microbial Drug Resistance. 2010;16(1).
 18. Bonnet R. Growing group of extended-spectrum β -lactamases: The CTX-M enzymes. Antimicrobial Agents and Chemotherapy. 2004;48:1-14.
 19. Réjiba S, Paola SM, Pablo P, Amel K. Emergence and dominance of CTX-M-15 extended spectrum beta-lactamase among *Escherichia coli* isolates from children. Microbial Drug Resistance. 2011;17(2).
 20. Ramdani-Bouguessa N, Mendonc N, Leitaño J, Ferreira E, Tazir M, Cania M. CTX-M-3 and CTX-M-15 extended-spectrum β -lactamases in isolates of *Escherichia coli* from hospital in Algiers, Algeria. Journal of Clinical Microbiology. 2006;44:4584-4586.
 21. Kiratisin P, Apisanthanarak A, Laespira C, Saifon P. Molecular characterization and epidemiology of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrobial Agents Chemother. 2008;52:2818-2824.
 22. Lartigue MF, Zinsius C, Wenger A, Bille J, Poirel L, Nordmann P. Extended-spectrum β -lactamases of the CTX-M type now in Switzerland. Antimicrobial Agents Chemother. 2007;51:2855-2860.
 23. Bindayna K, Khanfar HS, Senok AC, Botta GA. Predominance of CTX-M genotype among extended-spectrum beta-lactamase isolates in a tertiary hospital in Saudi Arabia. Saudi Medical Journal. 2010;30: 859-863.
 24. Haenni M, Saras E, Métayer V, Doublet B, Cloeckaert A, Madec JY. Spread of blaTEM-52 gene is mainly ensured by Inc11/ST36 plasmids in *Escherichia coli* isolated from cattle in France. Journal of Antimicrobial Chemotherapy. 2012;67: 2774-2776.
 25. Bertrand S, Weill FX, Cloeckaert A, Vrints M, Mairiaux E, Praud K, Dierick K, Wildemaue C, Godard C, Butaye P, Imberechts H, Grimont PA, Collard JM. Clonal emergence of extended-spectrum β -lactamase (CTX-M-2)-producing *Salmonella enterica* serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). Journal of Clinical Microbiology. 2006;44: 2897-2903.
 26. Dutin L, Irwin R, Finley R, Ng LK, Avery B, Boerlin P, Bourgault AM, Cole L, Daignault D, Desruisseau A, Demczuk W, Hoang L, Horsman GB, Ismail J, Jamieson F, Maki A, Pacagnella A, Pillai DR. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerging Infectious Disease. 2010;16:48-54.
 27. Canton R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM. Prevalence and spread of extended-spectrum β -lactamase producing Enterobacteriaceae in Europe. Clinical Microbiology Infection. 2008 ;14(Suppl 1): 144-153.
 28. Felix R, Viktoria A, Günter K. Extended-spectrum β -Lactamase and AmpC producing enterobacteria in healthy broiler

- chickens, Germany. *Emerging Infectious Diseases*. 2013;19(8).
29. Horton RA, Randall LP, Snary EL, Cockrem H, Lotz S, Le Port H, Duncan D, Rabie A, McLaren I, Watson E, La Ragione RM, Coldman NG. Fecal carriage and shedding density of CTX-M extended-spectrum β -lactamase-producing *Escherichia coli* in cattle, chickens, and pigs: Implications for environmental contamination and food production. *Applied and Environmental Microbiology*. 2011;77:3715–9.
30. Marshall BM, Levy SB. Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Review*. 2011;24: 718–33.
31. Madec JY, Poirel L, Saras E, Gourguechon A, Girlich D, Nordmann P, Haenni M. Non-ST131 *Escherichia coli* from cattle harbouring human-like *bla*CTX-M-15-carrying plasmids. *Journal of Antimicrobial Chemotherapy*. 2012;67:578-581.
32. Cloeckaert A, Praud K, Lefevre M, Doublet B, Pardos M, Granier SA, Brisabois A, Weill FX. Inc1 plasmid carrying extended-spectrum- β -lactamase gene *bla*CTX-M-1 in *Salmonella enterica* isolates from poultry and humans in France, 2003 to 2008. *Antimicrobial Agents Chemotherapy*. 2010;54:4484-4486.
33. Diwan V, Tamhankar AJ, Khandal RK, Aggarwal M, Sen S. Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. *BMC Public Health*. 2010;10:414.
34. Reinthaler FF, Feierl G, Galler H, Haas D, Leitner E, Mascher F, Melkes A, Posch J, Winter I, Yarfel G, Marth E. ESBL-producing *E. coli* in Austrian sewage sludge. *Water Research*. 2010;44:1981-1985.
35. Nagulapally SR, Ahmad A, Henry A, Marchin GL, Zurek L, Bhandari A. Occurrence of ciprofloxacin-, trimethoprim-sulfamethoxazole, and vancomycin-resistant bacteria in a municipal wastewater treatment plant. *Water Environment Research*. 2009;81: 82–90.
36. Vishal D, Chandran SP, Tamhankar AJ, Lundborg CS, Macaden R. Identification of extended-spectrum β -lactamase and quinolone resistance genes in *Escherichia coli* isolated from hospital wastewater from central India. *Journal of Antimicrobial and Chemotherapy*. 2012;67:857–859.

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