Original Article

Prevalence and characterization of extended spectrum beta-lactamase-producing *Enterobacter cloacae* strains in Algeria

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Abstract

Introduction: Expended spectrum β-lactamase (ESBL)-producing *Enterobacter cloacae* is an important nosocomial pathogen. In this study, the prevalence and the molecular epidemiology of ESBL producing *E. cloacae* strains isolated from various hospitals in Annaba, Algeria were investigated.

Methodology: The study involved 63 isolates of *E. cloacae* obtained during 2009 at the four hospitals in Annaba. The detection of ESBL was performed using the double-disk synergy test and the combined disk test. Minimum inhibitory concentrations (MICs) were determined using the agar dilution method. The presence of $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM}, and bla_{DHA} β-lactamase genes was evaluated by PCR, and genomic typing was determined by pulsed-field gel electrophoresis (PFGE) analysis. The clinical and microbiological data were entered into the EpiI Info database.

Results: Thirty isolates (47.6%) had an ESBL phenotype. $Bla_{\text{CTX-M}}$ group1 (76%); bla_{TEM} (70%) were the most prevalent, followed by bla_{DHA} (16.6%) and bla_{SHV} (10%). Eighteen strains expressed at least two *bla* genes. MICs revealed a high level of resistance to cefotaxime, ceftazidime, and cefepime. PFGE revealed an epidemic clonal dissemination of these isolates. Various risk factors associated with the occurrence of ESBL-producing *E. cloacae* were detected.

Conclusions: A higher frequency of ESBL-producing isolates and a diversity of β-lactamases were detected among ESBL-producing *E. cloacae*; these resulted from an epidemic clonal dissemination and high transference of ESBL genes between bacteria in hospital settings. Strict measures will be required to control the further spread of these pathogens in hospital settings.

Key words: *Enterobacter cloacae*; ESBL; clonal dissemination; risk factors

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Introduction

Infections caused by expended spectrum β-lactamase (ESBL)-producing bacteria result in increased mortality in hospitalized patients [1]. Invasive procedures, admission to intensive care units, and previous use of antibiotics are the most common risk factors for ESBL-producing bacteria [2]. *Enterobacter* species, particularly *E. cloacae* and *E. aerogenes,* are important nosocomial pathogens responsible for various infections [3-5] and are therefore under intensive selective pressure from broad-spectrum β-lactam use [6].

Various kinds of β-lactamases have been described in *E. cloacae*. The chromosomally encoded cephalosporinase is common to all strains of *E. cloacae*. In addition to the class C cephalosporinase, other β-lactamases have been reported in this species, including ESBL enzymes [7].

These ESBL genes are known to spread to other members of the *Enterobacteriaceae* and have been classified into nine distinct structural and evolutionary families based on their amino acid sequences [8]. TEM and SHV enzymes form major families. In the past decade, CTX-M type has emerged in many countries [9]. Currently, the CTX-M family includes more than 130 β-lactamases, which are grouped on the basis of sequence similarity into five distinct clusters (subtypes): CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (www.lahey.org/Studies/).

The present study explored the prevalence and molecular epidemiology of ESBL-producing *E. cloacae* strains isolated from various hospitals in

Annaba, Algeria. An attempt was also made to detect the resistance of ESBL strains to other antimicrobial groups and to analyze various risk factors associated with the occurrence of infections by ESBL-producing strains.

Methodology

Bacterial isolates

A total of sixty-three consecutive and non-duplicate clinical isolates of *E. cloacae* were examined. These isolates were collected over a period of one year (between January 2009 and December 2009) from four hospitals (Dorban, Ibn Rochd, Ibn Sina, and Sainte-Thérèse hospitals) in Annaba, Algeria. These isolates were collected from different patients and distributed among several wards (intensive care unit, endocrinology, pediatric, urology, pneumology, orthopedic, hematology, and general medicine). The isolates included 22 from urine (35%), 20 from blood culture (31.7%), 13 from pus (20.6%), 5 from protected distal bronchial (8%), 2 from plural liquid (3.1%) and 1 from nasal swabs (1.6%).

Antimicrobial susceptibility testing and screening for ESBL

Antimicrobial drug susceptibility was determined using the disk diffusion method on Mueller-Hinton (MH) agar plates (Bio-Rad, Marnes-la-Coquette, France) as recommended by the Clinical and Laboratory Standards Institute (CLSI) [10]. The following antimicrobial agents (Bio-Rad) were tested: amoxicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (10 µg), aztreonam (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), pefloxacin (5 µg), ofloxacin (5 µg), norfloxacin (10 µg), gentamicin (10 µg), tobramycin (10 µg), kanamycin (30 µg), amikacin (30 µg), netelmicin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), fosfomycin (200 µg), chloramphenicol (30 µg), and tetracycline (30 µg).

ESBL production was screened by double disk with a synergy test and agar diffusion test between a central amoxicillin clavulanic acid disk and a third-generation cephalosporin (cefotaxime, ceftazidime, cefepime, cefpirome) or a monobactam (aztreonam), placed at a distance of 30 mM (center to center) as previously described [11]. The standard strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls of ESBL production.

Minimal inhibitory concentrations of β-lactams (cefotaxime, ceftazidime, cefepime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, imipenem) were determined using the agar dilution method according to CLSI guidelines [10] for ESBL-carrying strains.

Preparation of DNA template for PCR

Total DNA was extracted by suspending a few colonies of overnight culture of E. cloacae isolates growing on Luria Bertani agar (Bio-Rad, Marnes-la-Coquette, France) in 500 µL of DNase- and RNase-free water (Invitrogen, Paisley, UK). The suspension was boiled at 100°C for 10 minutes in thermal block (Polystat 5, Bioblock Scientific, France), then centrifuged at 19000 x g for 5 minutes. An aliquot of 1 µL of the supernatant was used as the DNA template for PCR.

Detection of β-lactamase-encoding genes

E. cloacae isolates were screened by PCR for the following β-lactamase encoding genes: $bla_{CTX\text{-}M}$

Table 1. Primers used for detection of SHV, CTX-M, TEM, and DHA genes

Genes	Primer [a]	Nucleotide Sequence (5'→3')	Amplicon size
$bla_{CTX\text{-}M\ group\ 1}$	CTX-M-1(+)	GGTTAAAAAATCACTGCGTC	863bp
	CTX-M-1(-)	TTGGTGACGATTTTAGCCGC	
$bla_{CTX\text{-}M\ group\ 2}$	CTX-M-2(+)	ATGATGACTCAGAGCATTCG	865bp
	CTX-M-2(-)	TGGGTTACGATTTTCGCCGC	
$bla_{CTX\text{-}M\ group\ 9}$	CTX-M-9(+)	ATGGTGACAAAGAGAGTGCA	869bp
	CTX-M-9(-)	CCCTTCGGCGATGATTCTC	
bla_{SHV}	Os-5	CGCCGGGTTATTCTTATTTGTCGC	795bp
	Os-6	CGCCGGGTTATTCTTATTTGTCGC	
bla_{TEM}	a-216	ATAAAATTCTTGAAGACGAAA	1079bp
	a-217	GACAGTTACCAATGCTTAATCA	
bla_{DHA}	dhaM(+)	AACTTTCACAGGTGTGCTGGGT	405bp
	dhaM(-)	CCGTACGCATACTGGCTTTGC	

[a] + Primer forward; - Primer reverse

phylogenetic lineage groups 1, 2 and 9, bla_{TEM}, bla_{SHV}, and bla_{DHA} as described previously [12]. All primers used in this work are shown in Table 1.

Amplification reactions were performed in a volume of 50 µL containing 2 µL of DNA template, 2.5mM $MgCl_2$, 0.4 µM of each forward and reverse primers, 100 µM of each dNTP, and 2 units Taq DNA polymerase (Promega, Madison, USA) in 1 × PCR buffer provided by the manufacturer.

Cycling parameters included five minutes of denaturation at 95°C, followed by 30 cycles of denaturation (95°C for one minute), annealing (60°C for one minute for CTX-M and SHV, 52°C for one minute for TEM, 64°C for one minute for DHA), and extension (72°C for one minute), ending with a final extension period of 72°C for seven minutes.

The known β-lactamase-producing strains *E. coli* U2A1790 (CTX-M-1), *E. coli* U2A1799 (CTX-M-9), *Salmonella* spp. U2A2145 (CTX-M-2), *Salmonella* spp. U2A1446 (TEM-1 and SHV-12), and *K. pneumoniae* U2A 2240 (DHA-1) were used as positive controls. *E. coli* $K_{12}J_5$ strain was used as a negative control.

PCR products were detected on 1.5% agarose gel (FMC Bioproduct, Rockland, USA) after ethidium bromide staining and UV illumination, photographed with an Olympus digital camera, and analyzed using Digi-Doc-it software (UVP, Upland, USA).

Genetic relationships

Genetic relationships of the ESBL-producing *E. cloacae* were assessed using PFGE after digestion with XbaI. The PFGE analysis of XbaI-digested genomic DNA was performed using a CHEF-DRIII apparatus (Bio-Rad, Hercules, USA) according to the instruction manual. Bacterial DNA and XbaI digestion was prepared as previously described [13]. Fragment separation was done in 1% agarose gel with the following conditions: 6 V/cM for 22 hours with switch times of 5-30 seconds in Tris-borate-EDTA buffer pre-chilled to 12°C.

The similarity of the PFGE banding patterns was analyzed by the Dice coefficient, and the data obtained were analyzed by the unweighted pair group method with arithmetic average (UPGMA) clustering using the Pearson correlation coefficient (Biochemistry and Biotechnology Department, Rovira i Virgili University, Tarragona, Spain) (http://genomes.urv.cat/UPGMA/index.php).

Interpretation of chromosomal DNA restriction patterns was based on the criteria of Tenover et al. [14]. Briefly, strains showing more than three DNA fragment variations and a similarity of < 80% at dendrogram analysis were considered to represent different PFGE types, while one- to three-fragment differences and a similarity of > 80% upon dendrogram analysis were considered to represent PFGE pattern subtypes.

Statistical analysis

Several epidemiological data were sought systematically in the clinical records of patients infected with ESBL-producing *E. cloacae*: age, sex, wards, site of sampling, nature of infection, length of stay, first prescription of antibiotics, history of hospitalization in the last year, mode of admission (direct or transfer), field of patient and often underlying pathology, and number of invasive procedures. A descriptive analysis using Epi Info version 6 software (CDC, Atlanta, USA) was performed. All data were recorded using Excel (Microsoft, New York, USA), and the statistical analysis was performed using Epi Info version 6 software.

Results

A total of 63 patients were included in this study. Thirty (47.6%) patients were enrolled as ESBL-producing *E. cloacae* strains, and 33 (52.4%) patients were included as non-ESBL.

The median age was 41 years (range = 9 days to 73 years), and 63.3% were male. The average length of hospitalization was 13.2 days (range = 6 days to 29 days). Patient characteristics and laboratory data are described in Table 2.

There were many hospitalization wards with patients carrying bacteria-producing ESBLs during the study period. The wards were sorted according to the number of reported cases in each wards: medical wards (18/30), intensive care units (8/30), surgical wards (3/30), and emergency wards (1/30). The origin of these patients before admission in the wards (Table 2) is distributed between intensive care units (9/30), home (7/30), other hospitals (3/30), and others wards (11/30).

Among the 63 collected isolates, 51 isolates were resistant to third-generation cephalosporins. Phenotypic confirmatory methods detected ESBL production in 30 isolates (47.6%; Table 3). MICs of ESBL-producing isolates ranged from 64 to 2048 µg/mL for cefotaxime, and from 64 to 1024 µg/mL for both ceftazidime and cefepime.

Table 2. Characteristics of patients infected with ESBL-producing *E. cloacae*

Characteristic	Number of patients infected	Frequency (%)
Age		
Mean	41	-
Gender		
Female	11	36.66
Male	19	63.33
Sex-ratio	0.57	
Population		
Adult	21	70
Child	06	20
Newborn	03	10
Duration of hospitalization	13.2	
Origin		
Home	07	23.33
Reanimation ward	09	30
Other wards	11	36.66
External hospitals	03	10
Previous hospitalization (< 1 an)	25	83.33
Coexisting diseases:		
Trauma	03	15.78
Transplantation	02	10.5
Diabetes	06	31.5
Neoplasia	03	10.5
Hepatobiliary disease	02	31.5
Other diseases	03	10
Risk factor		
Intubation	06	20
Catheter	04	13.33
Urinary catheter	21	70
Nasogastric intubation	06	20
Surgery	11	36.6
Other	04	13.33
Previous antibiotic use	23	6.66
Third generation cephalosporins	04	19.4
Third generation cephalosporins + aminglycoside	11	52.3
Third generation cephalosporins + Fluoroquinolone	02	9.5
Fluoroquinolone	02	9.5
Other	04	19.4
Patients without antibiotic presumptive	07	23.33
Monomicrobial infection	22	73.33
Polymicrobial infection	08	26.66
Klebsiella pneumoniae	02	25
Pseudomonas aeruginosa	03	37.5
Staphylococcus aureus	03	37.5

Table 3. Characteristics of ESBL-producing *E. cloacae* detected in Annaba hospitals

Code	Ward	Source	MIC (mg/L)						Resistance to antibiotics other than Beta-lactam	β-lactamase type	PFGE type
			CTX	CAZ	FEP	CTX/Ac	CAZ/Ac	IMP			
E1	Paediatric ICU	Protected distal bronchial	2048	256	256	0.03	2	0.125	Na, PEF, OFX, NOR, CIP, SXT, C, Tet, KTGNt	CTX-M, TEM	
E11	Endocrinology	Pus	2048	256	256	0.03	2	0.125	Na ,PEF,OFX ,NOR, CIP ,SXT, C, Tet	CTX-M, TEM	
E5	Surgical ICU	Protected distal bronchial	2048	256	256	0.03	2	0.125	SXT, C ,Tet ,KTGNt	CTX-M, TEM	CE1
E8	Orthopaedic	Pus	2048	256	256	0.03	2	0.125	Na, PEF ,OFX, NOR ,CIP, SXT, C, Tet, FOS, KTGANt	CTX-M ,TEM	
E18	Endocrinology	Pus	32	64	64	0.06	0.5	0.06	Na ,PEF ,OFX ,NOR ,CIP ,SXT, C, Tet ,KTGANt	TEM	
E16	Infectious Diseases	Urine	1024	512	512	0.06	0.25	0.125	FOS, SXT, C, Tet	CTX-M, TEM	
E21	Endocrinology	Pus	512	1024	512	0.125	16	0.06	FOS, SXT, C, Tet, KTGANt	CTX-M, TEM	
E25	Infectious Diseases	Urine	512	104	512	0.125	16	0.006	Na, SXT, C ,Tet ,KTGNt	CTXM, TEM , DHA	
E20	Paediatric	Urine	512	1024	512	0.125	16	0.125	FOS, SXT, C, Tet, KTGNt	TEM	CE2
E24	Infectious Diseases	Pus	512	1024	512	0.125	16	0.25	FOS, SXT, C, Tet ,KTGNt	TEM	
E26	Paediatric ICU	Protected distal bronchial	12	1024	512	0.12	16	0.006	Na, SXT, C, Tet, KTGNt	TEM	
E3	Surgical ICU	Protected distal bronchial	512	64	64	0.06	0.25	0.06	SXT, C,Tet	CTX-M	CE3
E30	Neonatology	Urine	2048	1024	1024	0.125	16	0.125	Na ,SXT, C, Tet, KTGANt	CTX-M, DHA	
E31	Neonatology	Pus	2048	1024	1024	0.125	16	0.125	NA, SXT, C Tet KTGANt	CTX-M, DHA	CE4
E32	Neonatology	Nasal	2048	1024	1024	0.125	16	0.125	NA ,SXT, C Tet KTGANt	CTX-M, DHA	
E15	Surgical ICU	Pus	1024	512	512	0.125	16	0.06	Na ,PEF, OFX ,NOR, CIP, SXT, C, Tet, KTGANt	CTXM	
E6	Emergency surgical	Plural liquid	2048	256	256	0.125	16	0.06	Na,PEF, OFX, NOR, CIP, SXT, C, Tet ,FOS, KTGANt	CTXM	CE5
E7	Surgical ICU	Pus	2048	256	256	0.125	16	0.06	Na,PEF,OFX ,NOR ,CIP, SXT, C ,Tet, FOS, KTGANt	CTXM	
E19	Paediatric	Urine	512	1024	512	0.05	16	0.125	Na, FOS, SXT, C ,Tet, KTGNt	SHV,TEM ,CTXM	
E12	Surgical ICU	Urine	2048	128	256	0 .03	2	0.03	Na ,PEF, OFX, NOR ,CIP, SXT, C, Tet ,FOS, KTGNt	CTX-M, TEM	CE6
E9	Orthopaedic	Pus	2048	128	256	0.03	2	0.03	SXT, C ,Tet, KTGNt	CTX-M, TEM	
E17	Paediatric	Pus	32	64	64	0.06	0.25	0.03	Na FOS SXT C	TEM	CE7
E29	Surgical ICU	Pus	>2048	1024	1024	0.125	16	0.125	Na PEF CIP FOS SXT C Tet KTGANt	CTXM, TEM, DHA	CE8
E2	Infectious Diseases	Pus	32	256	64	0.06	0.5	0.06	Na C Tet	TEM	CE9
E10	Infectious Diseases ICU	Blood culture	>2040	256	256	0.06	4	0.06	Na, SXT, C ,Tet, KTGANt	CTX-M	CE10
E13	Infectious Diseases	Urine	>2040	128	256	0.125	4	0.125	Na, SXT ,C ,Tet	CTX-M	CE11
E28	Paediatric	Urine	>2048	128	256	0.06	0.5	0.125	FOS, SXT, C,Tet	CTX-M, TEM	CE12
E22	Endocrinology	Pus	256	256	256	0.06	0.25	0.25	Na, SXT, C, Tet ,KTGNt	TEM, SHV	CE13
E23	Infectious Diseases	Urine	2048	256	256	0.12	4	0.25	FOS, SXT, C, Tet ,KTGNt	CTX-M, TEM	
E14	Urology	Urine	>2040	512	512	0.25	2	0.125	Na,SXT, C ,Tet	SHV, TEM, CTX-M	CE14

CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; CTX/Ac, cefotaxime/clavulanic acid; CAZ/Ac, ceftazidime/clavulanic acid; IMP, imipenem; NA,nalidixic acid; PEF,pefloxacin; OFX, ofloxacin; NOR, norfloxacin; CIP,ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; Tet, tetracycline; FOS, fosfomycin; C, chloramphenicol; A, amikacin; G, Gentamicin; Nt, netelmicin; T, tobramycin; K, kanamycin

All isolates were susceptible to cefotaxime/clavulanic acid (MICs 0.03-0.125 µg/mL), ceftazidime/clavulanic acid (MICs 0.25-16 µg/mL), and imipenem (MICs 0.03–0.25 µg/mL). ESBL production was associated with aminoglycosides resistance in 73.3% of cases. This co-resistance was presented in two phenotypes: Kanamycin, Tobramycin, Gentamicin, and Netelmicin resistance (KTGNt) and Kanamycin, Tobramycin, Gentamicin, Amikacin, and Netelmicin resistance (KTGANt). The overall resistance to various antibiotics was as follows: chloramphenocol 100%, tetracycline 100%, cotrimoxazole 96.6%, and quinolones 33.3%. All isolates were sensitive to colistin and imipenem.

The total DNA isolated from 30 ESBL-producing organisms were subjected to PCR using TEM, SHV, and CTX-M specific primers. Two or more genes for ESBL were present in 15 (50%) of 30 ESBL typeable isolates, bla_{TEM} + $bla_{CTX-M,}$ being the most common combination (33.3%) followed by bla_{TEM} + bla_{SHV} + bla_{CTX-M} (6.6%), and bla_{TEM} + bla_{SHV} (3.3%). Among the isolates harbouring a single ESBL gene, bla_{CTX-M} was present in 30 per cent and bla_{TEM} in 20 per cent. All CTX-M enzymes in *E. cloacae* belonged to the CTXM-1 cluster.

The cephalosporinase DHA type was found in five *E. cloacae*. This gene has been presented with the following combinations: bla_{DHA-1} + bla_{CTX-M} (n = 3) and bla_{DHA}+ bla_{CTX-M}+ bla_{TEM} (n = 2).

PFGE analysis revealed fourteen different clonal types (Figure 1) among the 30 ESBL-producing *E. cloacae* isolates. Eight of these clones were represented by a single isolate each (isolate E3, clone CE3; isolate E17, clone CE7; isolate E29, clone CE8; isolate E2, clone CE9; isolate E10, clone CE10; isolate E13, clone CE11; isolate E28, clone CE12, and isolate E14, clone CE14). The other six clones were represented by six (clone CE2), five (clone CE1), four (clone CE5), three (clone CE4) and two (CE6 and CE13) isolates, respectively (Table 2). Clone CE5 and CE1 were isolated from two different hospitals. In contrast, the same isolates of other clones were found in patients hospitalized in the same ward within a few months of each other, showing that an undetected limited outbreak of ESBL-producing *E. cloacae* had occurred in that unit.

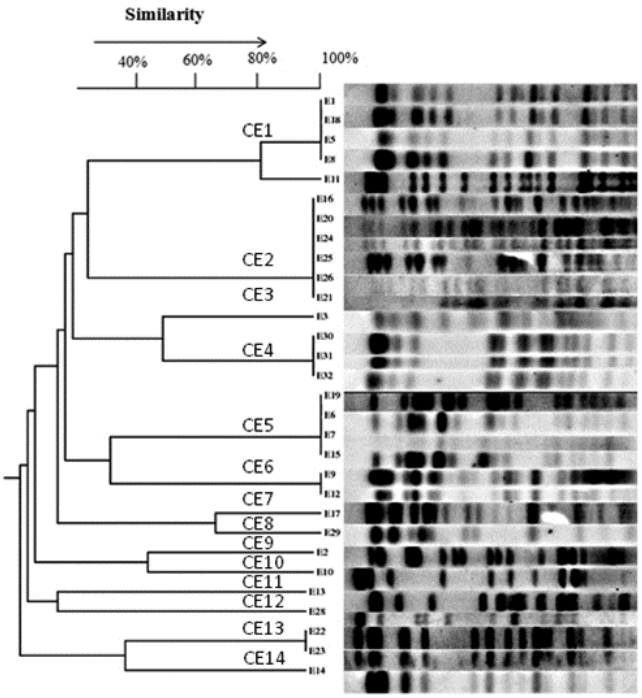
Various risk factors associated with the occurrence of ESBL-producing *E.cloacae* are shown in Table 2. When these factors were further subjected to multivariate analysis and antibiotic exposure (therapy), 17 of the 23 cases identified during the study period had been given a presumptive treatment of third-generation cephalosporins antibiotic, either alone (4/17) or combined with aminoglycoside (11/17) and fluroquinolone (2/17). Patients' use of a urinary catheter (70%) was found to be significantly associated with infections by ESBL-producing isolates.

Discussion

E. cloacae is frequently responsible for nosocomial infections, including urinary tract infections, pneumonia, and bloodstream infections [15-16]. In the current study, infections caused by ESBL-producing *E. cloacae* isolates were mainly urinary infections, followed by surgical wound infections and pulmonary infections. Theseresults are in accordance with the results reported in the literature [17].

This study found that the prevalence of ESBL-producing *E. cloacae* isolates in the Annaba hospitals was 47.6%, which was higher than that the prevalence rates found in Algerian hospitals (17.7%) [18] and in Korea (35.4%) [19]. *E. cloacae* with ESBL production has been reported to exceed 43% in countries such as Singapore, mainland China, and the Philippines [20]. The use of cefotaxime and, recently, ceftazidime in Algeria could partly explain this high percentage of ESBLs. Overall, the resistance patterns of ESBL-

Figure 1. Dendrogram and PFGE of *Xba*I-digested genomic DNAs from ESBL-producing *E. cloacae* collected in Annaba hospitals of Algeria



producing *E. cloacae* studied here were similar to those commonly described in other studies (*i.e*., the ESBL producers were resistant to different antibiotic families including β-lactams, fluoroquinolones, aminoglycosides, and trimethoprim/sulfamethoxazole) [18,21-23].

The CTX-M type was the most common ESBL in the Annaba hospitals, which was higher than that reported from Algerian hospitals (20%) [18]. CTX-M group 1 remains the only subgroup of CTX-M ESBLs found in Algeria with the predominance of CTX-M-3 and CTX-M-15 enzymes [18,24]. Encoding genes for the SHV family were detected in 10% of the isolates. Most enzymes from this group are ESBL, except for SHV-1 and SHV-11 [25-26]. However, the enzymes from this group cannot be confirmed to be ESBL-encoding genes, mostly in strains presenting more than one *bla* gene. On the other hand, the $bla_{\text{SHV-12}}$ gene was also detected in *E. cloacae* from Algerian hospitals [21]. The bla_{TEM} gene was identified in 70% of isolates, generally in association with SHV and/or CTX-M genes. Therefore, it was not possible to determine if they were responsible for the ESBL phenotype or not; they could be non-ESBL encoding genes associated with another gene expressing the extended-spectrum phenotype [27]. On the other hand, co-production of different ESBLs by a unique strain has been a common finding in several studies [18,25-26,28], so the carriage of two or three ESBL-encoding genes by the TEM-producing strains of this study could also have occurred.

In the present study, PFGE revealed fourteen different pulsotypes among the 30 ESBL-producing *E. cloacae* isolates. The services most affected by the clonal spread were infectious disease (6 clones), surgery (5 clones), and paediatrics wards (4 clones). These wards have patients with fragile host defences (36% of patients harboured a disease) receiving excessive manipulation and a high antimicrobial intake (74% of patients had received antibiotic treatment), which could have been predisposing factors for infections and the spread of resistance genes. A total of 46% of patients in the current study were already hospitalized in other wards and other hospitals; the movements of patients between different hospital wards must therefore also be considered.

In the univariate analysis, prolonged hospital stay, intensive care unit admission, urinary catheter use, and elective surgery were risk factors associated with ESBL strains. All these conditions are related to the severe conditions of the patients, as well as the use of broad-spectrum antibiotics. A previously published study verified irrelevant risk factors for *Enterobacter* bacteremia among other bacteria, but no comparison of different resistance profiles was made [29-30]. Chang *et al*. [31] showed that fourth-generation cephalosporin resistance was an independent risk factor of *Enterobacter* bacteremia. Prior antibiotic administration is a major factor for colonization and secondary infections with these multiple-antibiotic-resistant organisms. Clinicians are advised to avoid unnecessary administration of antimicrobial agents and to avoid unnecessary prolonged administration. For surgical prophylaxis, administration of antibiotics for longer than 24 hours is rarely justifiable. Education programs for hospital personnel about risk reduction in the transmission of *Enterobacter* species and other nosocomial pathogens should be implemented in hospitals. This is usually the responsibility of the infection control team.

Conclusion

In summary, the data obtained in this study showed that higher rates of ESBL-producing *E. cloacae* isolate in Annaba hospitals have resulted from an epidemic clonal dissemination in different hospital wards. Various risk factors associated with the occurrence of ESBL-producing *E. cloacae* were detected. Genes $bla_{\text{CTX-M}}$ and bla_{TEM} were detected in high frequency among ESBL-positive isolates, occurring alone or in combination, indicating the high transference of ESBL genes between bacteria in hospital settings. Strict measures will be required to control the further spread of these pathogens in hospital settings.

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