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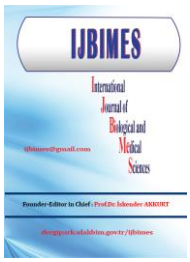

ResearchArticle

First characterization of β-lactamases among clinical isolates of enterobacteria in the intensive care unit of Laghouat Hospital, Algeria[#]

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Abstract: Extended-spectrum β-lactamases producing Enterobacteriaceae (ESBL-E) are emerging worldwide in hospitals and in the community. The aim of the present study was designed to characterize the extended-spectrum β-lactamase (ESBL) types in clinical isolates of Enterobacteriaceae and their clonal dissemination in the intensive care unit (ICU) of Laghouat hospital, Algeria. During the study period (from March 2011 to September 2014), 247Enterobacteriaceae were isolated from various clinical specimens. Fifty six (22.67%) Enterobacteriaceae isolates were found to ESBL-producers: (48.21%) *K. pneumonia*, (28.57%), *E. coli* and (23.21%) *E. cloacae*. All ESBL-producing isolates were multidrug resistant. The genetic analysis showed that all ESBL-producing Enterobacteriaceae isolates were produced CTX-M-15, whereas TEM-4 was detected in only one isolate with 01.78% (1/56). ERIC-PCR analysis showed that the isolates are genetically unrelated. Our results underline the need for continuous surveillance of the high prevalence and evolution of this CTX-M-15 type β-lactamase in the intensive care unit of Laghouat hospital, Algeria.

1. Introduction

Extended-spectrum β-lactamases (ESBLs) producing Enterobacteriaceae are responsible for many local, national and international outbreaks which frequently originated in the intensive care unit (ICU). Infections by these strains represent an increased risk of therapeutic failure and are associated with longer duration of hospital stay and higher hospital charges [1]. They are enzymes often clavulanate-susceptible that can hydrolyze oxyimino-cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefepime) and monobactams, but not cephamycins and carbapenems [2]. These ESBLs are class A/group 2 be β-lactamases. A majority of them are derivatives of TEM-1 (a plasmid-mediated β-lactamase of *Escherichia coli*) or SHV-1 (a chromosomal β-lactamase of *Klebsiella pneumoniae*) [3]. Recently, a new family of extended-spectrum β-lactamases called CTX-M that preferentially hydrolyzes cefotaxime has emerged. It has been found in isolates of *Salmonella enterica* serovar, *Typhimurium*, *E. coli* mainly and other species of *Enterobacteriaceae* [4]. Currently, CTX-M-15 types ESBLs are the most widespread CTX-M enzyme worldwide. Many reports have documented the emergence of ESBL producing Enterobacteriaceae. In Algeria, CTX-M-type ESBLs were first detected in 2005 from Bejaia, east of Algeria where outbreaks of CTX-M-15 and CTX-M-3 producing Enterobacteriaceae isolates have been described in two hospitals [5].

However, various other types of ESBLs enzymes: SHV-11, SHV-12, SHV-28, SHV-98 ; SHV-99 ; SHV-100, SHV-110,TEM-4, TEM-48, TEM-110, TEM-188,VEB-1, PER-1, CTXM-3, CTXM-14, CTX-M-15, CTX-M-28 have been reported in different Algerian studies with CTX-M-15 being the most prevalent [6]. The aim of the present study was designed to characterize the extended-spectrum β-lactamase (ESBL) types in clinical isolates of

Enterobacteriaceae isolated in the intensive care unit (ICU) of Laghouat hospital, southern region of Algeria, and to investigate their antibiotic susceptibility and their clonal dissemination.

2. Materials and methods

2.1. Clinical isolates

Between March 2011 and September 2014, a total of 247 clinical isolates were isolated from 212 patients admitted in the intensive care unit (ICU) of Laghouat hospital, south of Algeria. These isolates were obtained from various clinical specimens; mainly from: urine, blood, tracheal aspirate and catheters. All Enterobacteriaceae isolates were identified using the API 20E System (bioMérieux, Marcy l'Etoile, France).

2.2. Antimicrobial susceptibility testing and ESBL detection

The antimicrobial susceptibility was tested on Mueller-Hinton agar by the standard disk diffusion method recommended by the Antibiogram Committee of the French Society for Microbiology (CA-SFM) [7]. The isolates were screened for ESBL production by a double disk synergy test (DDST) as described previously by Jarlier et *al*. [8]. *E. coli* ATCC 25922 was used as quality control strains.

2.3. Characterization of genes encoding ESBLs

DNA of clinical isolates was extracted by the boiling method and the extracted DNA was stored at –20°C for molecular amplification.

The identification of ESBL genes was carried out by a Polymerase Chain Reaction (PCR) [9]. These ESBL-encoding genes were identified using specific primers for the $bla_{\text{CTX-M}}$ and bla_{TEM}. The details of the operating conditions and annealing temperatures were performed to all ESBL producing isolates as previously described [10], and gel -electrophoresis of the PCR products was performed on agarose gel 1%. The amplicons of ESBLs producers were sequenced with the dideoxy chain termination method, in GATC Biotech AG (European Custom Sequencing Centre, Gottfried-Hagen-Stra ße 20, 51105 Köln). The nucleotide sequences were analyzed using the Codon Code Aligner software and were compared to sequences available at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

2.4. Clonal analysis by ERIC-PCR

The genetic diversity of the ESBL-producing Enterobacteriaceae isolates was examined by the ERIC-PCR (enterobacterial repetitive intergenic consensus PCR) method as described previously [10]. The primer was used for this amplification is ERIC-2: 5'AAGTAAGTGACTGGGGTGAGCG3'. Pattern profiles were considered different when at least one band differed.

2.5. Mating experiments and plasmids analysis

Mating experiments were performed with in vitro-obtained rifampin-resistant mutants of *E. coli* C600 (recipient strain) as previously described [11]. All transconjugants were selected on Mueller Hinton agar (Oxoid) containing rifampicin (300µg/L) and cefotaxime (1µg/L), and they were subjected to antimicrobial susceptibility testing and double-disk synergy test. Plasmid DNA extraction of the different clinical isolates and their transconjugants was performed according to the protocol determined by Kado and Liu [12].

3. Results

During the study period, 247 non-repetitive clinical isolates of Enterobacteriaceae were isolated from 212 patients admitted in the intensive care unit (ICU) of Laghouat hospital, Algeria.

Among these, fifty six (22.67%) isolates were identified to ESBLs producers by a double disk synergy test (DDST), with the predominant specie was *K. pneumoniae* (48.21%), and followed by *E. coli* (28.57%) and *E. cloacae* (23.21%) (Table 1).

Table 1. *Distribution of extended-spectrum β-lactamase-producing Enterobacteriaceae by species and years.*

ESBL isolates	2011		2012		2013		2014	
	%	(n)	%	(n)	%	(n)	%	(n)
Klebsiella pneumoniae	14.28 %	(2/14)	14.28%	(3/21)	29.03%	(9/31)	46.42%	(13/28)
Escherichia coli	19.04%	(4/21)	10.34 %	(3/29)	17.64%	(6/34)	12%	(3/25
Enterobacter cloacae	37.5 %	(3/8))	14.28%	(1/7)	35.29%	(6/17)	25%	(3/12
Total	20.93%	(9/43)	12.28%	(7/57)	25.6%	(21/82)	29.23%	(19/65)

The majority of the clinical isolates were from: urine (35.71%), catheters (28.57%), tracheal aspirate (25%) and blood (10.71%).

Antimicrobial susceptibility analyses were showed that the all clinical isolates were resistant to the following β-lactams: Amoxicillin, Ticarcillin, Piperacillin, Cefalotin, Amoxicillin/clavulanic acid, Ticarcilline/clavulanic acid, Cefotaxime, Ceftazidime, Aztreonam, Cefepime, Cefpirome, Cefuroxime, and Cefixime (Figure 1). In addition, All ESBL-producing isolates were also multidrug-resistant and most of them were resistant: 100% to kanamycin, chloramphenicol and sulfonamide. Whereas 71.43% were resistant to gentamycin, 87.5% to tobramycin, 58.92% to ofloxacin, 32.14% to ciprofloxacin and 92.86% to trimethoprime. However, all isolates were susceptible to imipenem, colistin and fosfomycin.

The PCR and sequence analysis were revealed the presence of the CTX-M-15 types in all fifty six ESBL-producing Enterobacteriaceae, whereas the $bla_{\text{TEM-4}}$ was detected in a combination with $bla_{\text{CTX-M-15}}$ only in one clinical isolate of *E. coli* with 01.78% (1/56). ERIC-PCR analyses were revealed different restriction patterns for each isolate. In total, presence the 48 different patterns in 56 clinical isolates. Plasmid analyses were showed the presence of 1 to 11 plasmids of different sizes in clinical isolates with dissemination of the same large plasmid transferable a high molecular weight ≈130kb.

4. Discussion

In our study, the prevalence of ESBLs producers was found to be 22.67%. This is a high prevalence compared to many studies European countries [13]. Comparing this rate with other studies in Egypt and Asia where alarming high carriage rate were detected in these populations with 48.93% and 40% respectively [14-15].

Among Enterobacteriaceae isolated during the study period, *K. pneumoniae* was the most prevalent, with a proportion of ESBL producers was 48.21%, as observed in previous studies [16].

Generally, all ESBL-producing isolates in this study were multidrug-resistant to different antibiotics. Already, the third generation cephalosporins antibiotics and the remaining β-lactam antibiotics are by far the largest group of antibacterial agents used in clinical medicine and they are among the most frequently prescribed antibiotics worldwide. They account for approximately 50% of global antibiotic consumption and with a few notable exceptions they remain the cornerstone of antibiotic therapy for a wide variety of systemic infections caused by bacterial pathogens [17].

Consequently, effective antibiotic therapy for treating these infections is limited to a small number of drugs such as carbapenems and thus increasing the chance of resistance to carbapenems among the *Enterobacteriaceae*.

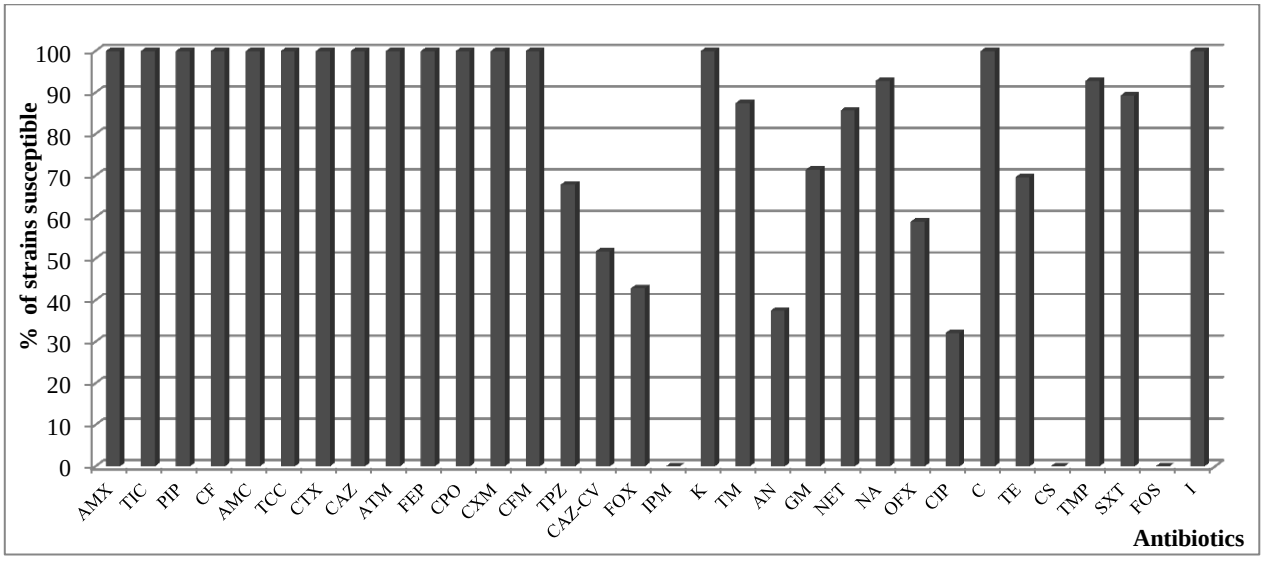

Figure 1. *Antimicrobial resistance rates of ESBL-Producing Enterobacteriaceae isolated in the intensive care unit. (AMX: amoxicillin, TIC: ticarcillin, PIP: piperacillin, CF: cefalotin, AMC: amoxicillin/clavulanic acid, TCC: ticarcilline/clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, ATM: aztreonam, FEP: cefepime, CPO: cefpirome, CXM: cefuroxime, CFM: Cefixime, TPZ: piperacillin + tazobactam, CAZ-CV : ceftazidime/clavulanic acid, FOX: cefoxitin, IPM: imipenem, K: kanamycin, TM: tobramycin, AN : amikacin, GM: gentamycin, NET: netilmicin, NA: nalidixic acid, OFX: ofloxacin, CIP: ciprofloxacin, C: chloramphenicol, TE: tetracycline, CS: colistin, TMP: trimethoprime, SXT: sulphamethoxazole/trimethoprim, FOS: fosfomycin, I: sulfonamide).*

In fact, all the 56 studied Enterobacteriaceae isolates in during the study period were producing CTX-M-15 ESBL, one of these were co-producing TEM-4 ESBL only in one clinical isolate of *E. coli* with 01.78%. The $bla_{CTX\text{-}M\text{-}15}$ gene are found mainly in *Enterobacteriaceae* and were recently named "plasmids of resistance responsible for outbreak" because of their capacity to acquire genes of resistance and to transfer among bacteria [18].
The ESBLs are mainly encoded by plasmids and mobile genetic elements such as integrons, insertion sequences, transposons and plasmids. These genetic elements are easily transferable to other bacteria such as those belonging to Enterobacteriaceae [19].
A study reported that the presence of a large plasmid transferable between ESBL-producing isolates. This property explains the easy dissemination of $bla_{\text{CTX-M}}$-harboring plasmids.

5. Conclusion

This is the first study from Laghouat hospital, Algeria that demonstrates such a high prevalence of CTX-M enzymes among Enterobacteriaceae strains isolated from intensive care unit (ICU). The $bla_{\text{CTX-M-15}}$ was the predominant ESBL gene in this hospital.

References

[1] Rodriguez-Villalobos H., M.J. Struelens "Extended-spectrum β-lactamases mediated bacterial resistance: Implications for the intensivist" Rev Réanimation. 15-3 (2006) 205-213

[2] Bush K., G.A. Jacoby "Updated Functional Classification of β-lactamases" Antimicrob Agents Chemother. 54 (2010) 969-976

[3] Bush K., G.A. Jacoby, A.A. Medeiros "A functional classification scheme for β-lactamases and its correlation with molecular structure" Antimicrob Agents Chemother. 39 (1995) 1211-1233

[4] Gazouli M., E. Tzelepi, A. Markogiannakis, N.J. Legakis, L.S.Tzouvelekis "Two novel plasmid-mediated cefotaxime hydrolyzing β-lactamases (CTX-M-5 and CTX-M-6) from *Salmonella typhimurium*" FEMS Microbiol Lett. 165 (1998) 289-293

[5] Touati A., S. Benallaoua, D. Forte, J. Madoux, L. Brasme, C. de Champs "First report of CTX-M-15 and CTX-M-3 β-lactamases among clinical isolates of Enterobacteriaceae in Béjaia, Algeria" J Antimicrob Agents. 27 (2005) 397-402

[6] Berrazeg M., M. Drissi, L. Medjahed, J.M. Rolain "Hierarchical clustering as a rapid tool for surveillance of emerging antibiotic resistance phenotypes in *Klebsiella pneumoniae* strains" J Med Microbiol. 62 (2013) 864-874

[7] CASFM "Antibiogram Committee of French Society for Microbiology. Communique 2010" Paris. France http: //www.sfm-microbiologie.org/UserFiles/files/casfm_2014.pdf.

[8] Jarlier V., M.H. Nicolas, G. Fournier, A. Philippon "Extended-broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns" Rev Infect Dis. 10 (1988) 867-878

[9] De Champs C., C. Chanal, D. Sirot, R. Baraduc, J.P. Romaszko, R. Bonnet, A. Plaidy, M. Boyer, E. Carroy, M.C. Gbadamassi et al "Frequency and diversity of class A extended-spectrum beta-lactamases in hospitals of the Auvergne, France: a 2 year prospective study" J Antimicrob Chemother. 54 (2004) 634-639

[10] Lagha N., D.E. Abdelouahid, H. Hassaine, F. Robin, R. Bonnet "First characterization of CTX-M-15 and DHA-1 β-lactamases among clinical isolates of *Klebsiella pneumoniae* in Laghouat Hospital, Algeria" Afr. J. Microbiol. Res. 8-11 (2014) 1221-1227.

[11] Sambrook J., E.F. Tritsch, T. Maniatis "Molecular cloning: a laboratory manual" Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 2nd ed (1989)

[12] Kado C.I., S.T. Liu "Rapid procedure for detection and isolation of large and small plasmids" J. Bacteriol (1981)

[13] Kaarme J., Y. Molin, B. Olsen, A. Melhus "Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in healthy Swedish preschool children" Acta Paediatr. 102 (2013) 655-660.

[14] Abdallah H.M., B.B. Wintermans, E.A. Reuland, A. Koek, N. al Naiemi, A.M. Ammar, et al "Extended-Spectrum β-Lactamase and Carbapenemase-Producing Enterobacteriaceae Isolated from Egyptian Patients with Suspected Blood Stream Infection" PLoS ONE 10-5 (2015) e0128120

[15] Lukac P.J., R.A. Bonomo, L.K. Logan "Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Children: Old Foe, Emerging Threat" Cli. Inf. Dis. Adv. CID : 1-10 (2015)

[16] Nedjai S., A. Barguigua, N. Djahmi, L. Jamali, K. Zerouali, M. Dekhil, M. Timinouni "Prevalence and characterization of extended spectrum β-lactamases in *Klebsiella-Enterobacter-Serratia* group bacteria, in Algeria" Med Mal Infect. 42 (2011) 20-29

[17] Pitout J.D. "Infections with extended-spectrum beta-lactamase producing Enterobacteriaceae: changing epidemiology and drug treatment choices" Drugs. 70 (2010) 313-333

[18] Coque T.M., A. Novais, A. Carattoli, L. Poirel, J. Pitout, L. Peixe, et al "Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15" Emerg Infect Dis. 14 (2008) 195-200

[19] Giedraitiené A., A. Vitkauskiené, R. Naginiené, A. Pavilonis "Antibiotic resistance mechanisms of clinically important bacteria" Medicina (Kaunas). 47 (2011) 137-146