EXTENDED SPECTRUM BETA-LACTAMASES: A MOLECULAR AND PHENOTIPIC STUDY

Daniel Estrada, Javier Zavala, Quintin Rascon, Blanca Rivera; Facultad de Ciencias Químicas, Universidad Autonoma de Chihuahua, Chihuahua, Chih; 31000; bchavira@uach.mx

ESBL, antibiotic resistance, CTX-M, TEM, SHV, beta-lactams, extended-spectrum beta-lactamases

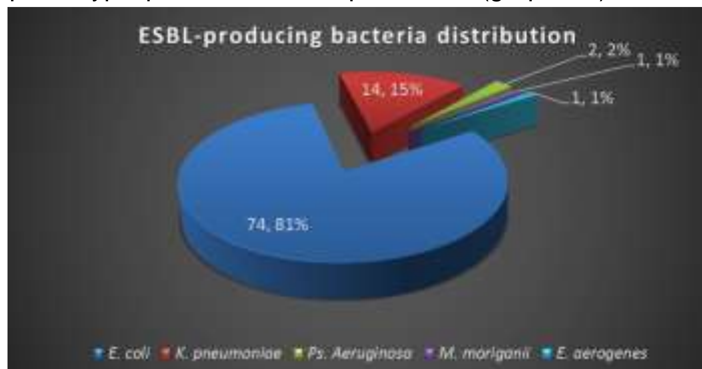
Introduction. Bacteria can resist antibiotic as a result of chromosomal mutation or inductive expression of a latent chromosomal gene or by exchange of genetic material though transformation, transduction, or conjugation by plasmids. Resistance can be transferred horizontally by plasmids or by chromosomally located conjugative transposons. Due to the alterations at the active site caused by specific point mutations, these extended spectrum-beta-lactamases (ESBL) are also available to hydrolyze oxyimino-beta-cephalosporins and aztreonam.

The objective of this study is to establish the phenotypic and molecular epidemiology from clinical isolates of this ESBL.

Methods. A total of 139 clinical isolates recovered in a period of 9 months were studied for presence of ESBL-encoding genes. Phenotypic methods used in this study were cefinase test, the positive cefinase isolates were tested by the double disc synergy test to confirm the ESBL production. Another method used was the boronic acid test to confirm the AmpC and ESBL coexistence.

ESBL-encoding genes analyzed in this study were CTX-M. TEM, SHV. Design of specific primers were performed by the multiple sequences alignment of the different genes the alignment was made using ClustalX. Once ESBL producing isolates were identified they were analyzed by PCR-DGGE.

Results. ESBL-encoding genes analyzed in this study were CTX-M. TEM, SHV. Design of specific primers were performed by the multiple sequences alignment of the different genes the alignment was made using ClustalX. Once ESBL producing isolates were identified they were analyzed by PCR-DGGE.
Results obtained shown that only 92 isolates were phenotypic positive to ESBL production (graphic 1).



Graphic 1: ESBL-producing bacteria distribution observed in the clinical isolates.

Coexistence of two different beta-lactam resistance genes were observed, broad spectrum beta-lactamases (AmpC) and ESBL, where were observed in the 88% of the isolates.

For PCR-based CTX-M identification was expected a 272 bp amplicon (figure1), for PCR-based TEM a 395 bp amplicon was expected (figure 2). Used primers contained a GC-pin to prevent premature denaturalization in the DGGE.

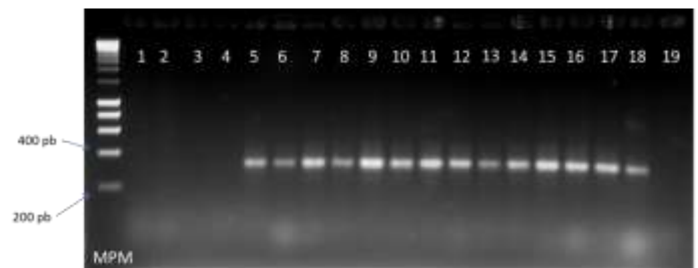


Figure 1: CTX-M PCR amplification. MPM: Hyperladder I, Lanes 1-18 containing clinical isolates DNA templates, Lane 19 negative control.

Figure 2: TEM PCR amplification. MPM: Hyperladder V, Lanes 2-18 containing clinical isolates DNA templates, Lane 1 negative control.

The CTX-M DGGE PCR-coupled experiment shown at least 6 different conserved sequences (figure 3).

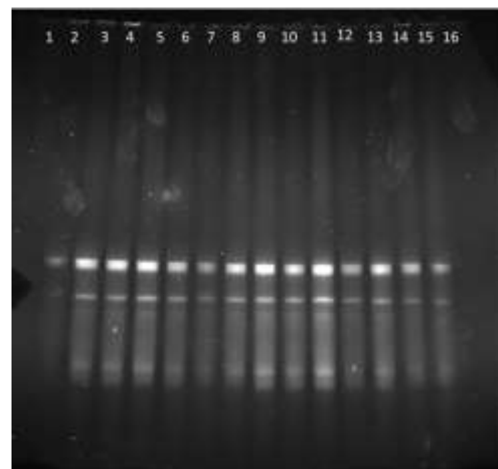


Figure 3: CTX-M PCR-positive samples DGGE. Lanes 1 and 16 were empty.

TEM DGGE PCR-coupled experiment shown more divergence **(figure 4)**.

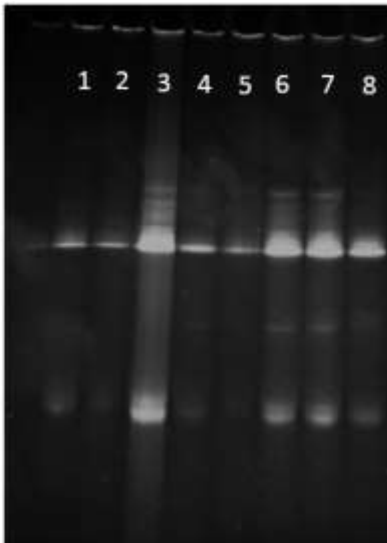


Figure 4: TEM PCR-positive samples DGGE.

DGGE analysis shown the existence of at least 4 different sequences for the CTX-M gene in the same isolate. For TEM gene 2 different sequences were common. Phylogenetic analyses has shown at least ten CTX-M genes non-related to CTX-M genes reported as see in the figure 5.

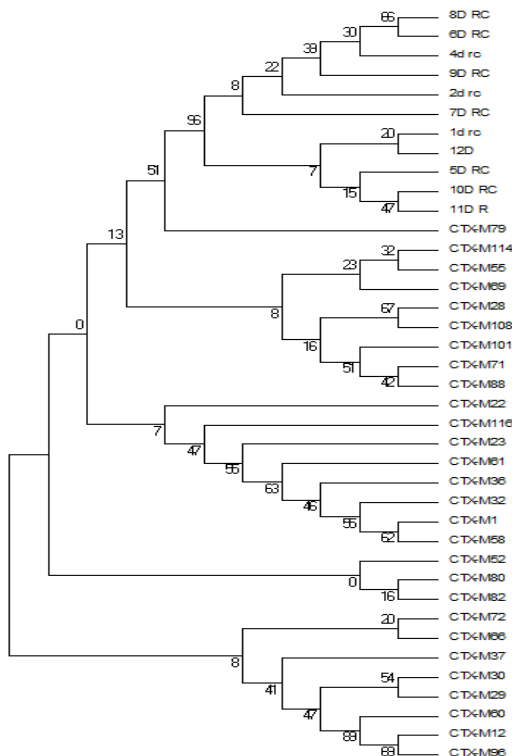


Figure 5. Maximum Likelihood tree obtained with retrieved CTX-M reported sequences and sequences obtained from the DGGE.

Conclusions. In Mexico, there is not an epidemiology study about ESBL distribution our data has demonstrated the CTX-M (81%) gene high prevalence and the existence of multiple CTX-M in the same isolate as well as in the case of the TEM genes. Also multiple ESBL-genes harboring bacteria were detected.

References.

1. Pleiss J. The Lac tamase E ngineering D atabase Institute of Technical Biochemistry, University of Stuttgart2011. The LacED integrates information on mutations, sequences, and structures of TEM and SHV ß-lactamases to assign new ß-lactamases, to identify inconsistencies in public databases, and to facilitate protein engineering. Databases of other lactamase classes will be added later.]. Available from: http://www.laced.uni-stuttgart.de/.
2. Canton R, Gonzalez-Alba JM, Galan JC. CTX-M Enzymes: Origin and Diffusion. Frontiers in microbiology. 2012;3:110. PubMed PMID: 22485109. Pubmed Central PMCID: 3316993.
3. Rossolini GM, D'Andrea MM, Mugnaioli C. The spread of CTX-M-type extended-spectrum beta-lactamases. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2008 Jan;14 Suppl 1:33-41. PubMed PMID: 18154526.
4. Gniadkowski M. Evolution and epidemiology of extended-spectrum beta-lactamases (ESBLs) and ESBL-producing microorganisms. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2001 Nov;7(11):597-608. PubMed PMID: 11737084.
5. Poirel L, Naas T, Nordmann P. Genetic support of extended-spectrum beta-lactamases. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2008 Jan;14 Suppl 1:75-81. PubMed PMID: 18154530.