

## RESUMEN

*Klebsiella pneumoniae* es una enterobacteria de importancia mundial en el ámbito de la salud debido a su rápida adquisición de resistencia a antimicrobianos, dicha cualidad junto con los mecanismos de transmisión de infecciones, pueden ser estudiados mediante herramientas de análisis de la variabilidad genética, que permiten conocer las relaciones evolutivas entre patógenos causantes de brotes en hospitales y en la comunidad y también establecer mecanismos de contención de infecciones. En el presente estudio, 64 aislados clínicos procedentes de infecciones sistémicas reportadas en dos hospitales de referencia del Ecuador, Hospital Carlos Andrade Marín y Hospital de Especialidades Eugenio Espejo fueron tipificados mediante la técnica MLST (Multi-Locus-Sequence-Typing). Se estandarizaron y se optimizaron ensayos de reacción en cadena de polimerasa (PCR) de 7 genes constitutivos *rpoB*, *gapA*, *mdh*, *infB*, *tonB*, *phoE* y *pgi* de *K. pneumoniae*. Los productos de PCR fueron secuenciados y una vez curadas las secuencias, fueron comparadas contra una base de datos de referencia (Instituto Pasteur) para obtener el perfil alélico y su correspondiente secuencia tipo. Mediante análisis bioinformáticos se identificaron complejos y grupos clonales, se construyeron árboles de mínima expansión, árboles filogenéticos y redes divididas. Se encontraron 28 secuencias tipo, 25 previamente reportadas y 3 no reportadas; agrupadas en 2 complejos clonales, 3 grupos clonales y 15 singletons. Al compararlas frente al árbol filogenético y red dividida, se obtuvieron 2 complejos clonales y 1 grupo clonal. El análisis genotipo-fenotipo permitió determinar que, CC2 y GC3 correspondían a aislados susceptibles, ST15 correspondió a un patrón BLEE y ST258 a un patrón CRE. Se concluye que en los hospitales no se evidencia brotes, sino infecciones individuales.

### **PALABRAS CLAVE:**

- **TIPIFICACIÓN DE SECUENCIAS DE LOCUS MÚLTIPLES**
- *Klebsiella pneumoniae*
- **RESISTENCIA BACTERIANA**
- **GENOTIPO VS FENOTIPO**

## ABSTRACT

*Klebsiella pneumoniae* is a health global importance Enterobacteria due to the fast resistance acquirement. This characteristic and transmission mechanisms of infections can be studied through analysis tools of genetic variability of this pathogen. That allows to know the evolutionary relationships between pathogens causing outbreaks in hospitals and community and establish infection containment mechanisms. In this study, 64 bloodstream clinical isolates were obtained from two reference hospitals, Carlos Andrade Marín Hospital and Eugenio Espejo Hospital. After DNA isolation, 7 genes Multilocus Sequence Typing system (MLST) was performed on the genetic material. Polymerase Chain Reaction (PCR) assays, corresponding to seven *Klebsiella pneumoniae* housekeeping genes, *rpoB*, *gapA*, *mdh*, *infB*, *tonB*, *phoE* and *pgi* were optimized and standardized. PCR products were then sequenced and once chromatograms were cured, they were compared to Bacterial Isolate Genome Sequence Database (BIGSdb), Pasteur Institute, with the purpose of getting corresponding allelic profile and type sequences. The obtained results identified groups and clonal complexes. Minimum spanning trees, phylogenetic trees and split networks were then built with the aid of bioinformatics software. Twenty-eight sequence types were identified. Twenty-five sequence types were already reported while the remaining three were non-reported. Sequence types were later clustered showing 2 clonal complexes, 3 clonal groups and 15 singletons. When phylogenetic trees and split networks were used, 2 clonal complexes and 1 clonal group were found. The genotype phenotype analysis allowed to identify that the isolates within CC2 and GC3 were susceptible, ST15 corresponded to an ESBL and ST258 to CRE pattern. With this information, it is possible to affirm that hospitals do not show outbreaks, instead they have individual infections.

### KEY WORDS:

- **MULTILOCUS SEQUENCE TYPING**
- *Klebsiella pneumoniae*
- **BACTERIUM RESISTANCE**
- **GENOTYPE VS PHENOTYPE**