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## **CHARACTERIZATION OF A LOCAL HYPERVIRULENT *Klebsiella pneumoniae* CLINICAL STRAIN BASED ON BIOMARKERS AND *Galleria mellonella* INFECTION MODEL**

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Hypervirulent *Klebsiella pneumoniae* (hvKp), first characterized in Taiwan in the 1980s, has emerged as a global health concern. While widespread in Asia, Europe, and the Americas, its occurrence in Argentina is relatively rare. It is responsible for liver abscesses in community-acquired infections and associated with metastatic complications like endophthalmitis and meningitis. The most common capsular serotypes associated with hvKp are K1 and K2. Within these serotypes, ST23 is the predominant sequence type for K1, while ST65, ST375, and ST86 are frequently observed in K2 strains. HvKp strains are characterized by the presence of several accessory virulence genes, including *rmpA* and *rmpA2*, which contribute to increased capsule expression and hypermucoviscosity, as well as *iuc* and *iro*, which are involved in aerobactin and salmochelin siderophore biosynthesis, respectively. While hypermucoviscosity (HMV) by a positive string test and a broad antimicrobial susceptibility profile are indicative of hvKp, molecular confirmation through the detection of characteristic virulence genes is essential. However, in vivo hvKp virulence studies for the circulating ST are scarce highlighting the need for further research. The aim of this study was to evaluate the virulence of the first clinical isolate of hvKp (K2/ST375) recovered from a liver abscess in our region, which has not been previously reported in Argentina. This strain has been characterized by the HMV phenotype and the presence of four virulence genetic markers *rmpA*, *rmpA2*, *iucA*, and *iroB* genes. We used the infection model of *Galleria mellonella* larvae with the hvKp ST375 strain and the standard strain *K. pneumoniae* ATCC 700603 (a hypovirulent standard strain), as an in vivo virulence study. These larvae are an alternative model of infection, with a myriad of advantages: maintenance and handling are quite easy, it is considered to be more ethically acceptable than other models and their immune system has a lot of similarities to those of mammals. A serial gradient of bacterium inoculum was used (105, 106 and 107 CFU) on fifteen larvae for each condition. We observed a dose-

dependent effect for hvKp as well as for Kp ATCC 700603 along 96 hs post-infection, suggesting an adequate range of inoculum used. In addition, we observed for 10<sup>6</sup> CFU a 100 % death of larvae infected with hvKp at 24hs, while c.a. 50 % survived when Kp ATCC 700603 was used. Thus, showing a virulence phenotype for hvKp strain as compared with the ATCC strain. Overall, these results show that the combination of clinical data, MHV phenotype, molecular diagnostic, and in vivo infection experiments enables a deeper characterization of a global emerging pathotype such as hvKp clones in our region.

Palabras clave: Hypervirulent *Klebsiella pneumoniae* - Liver abscesses - Virulence gene - Siderophores - *Galleria mellonella* infection model