

TITLE: VIRULENCE, ANTIMICROBIALS RESISTANCE OF *Klebsiella* spp. AND GENOMIC CHARACTERIZATION OF *Klebsiella pneumoniae* EXTENDED SPECTRUM β -LACTAMASES PRODUCERS ISOLATED FROM PSITTACINE BIRDS WITH RESPIRATORY DISEASE

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ABSTRACT

Psittacine birds are among the most seized bird species in São Paulo, commonly kept as pet. The maintenance of these birds in captivity may represent a zoonotic risk and contribute to the propagation of multiresistant and beta-lactamase extended-spectrum (ESBLs) enterobacteria, such as *Klebsiella* spp. The aim of this study was to identify and characterize strains of *Klebsiella* spp. isolated from respiratory secretions of 46 diseased psittacines, determining virulence and resistance profile to 15 antimicrobials. Isolation was performed on MacConkey agar with incubation at 37°C for 24 h. The colonies were identified by MALDI-TOF MS. The antibiotic susceptibility was determined by the agar diffusion method. Amplified spectrum beta-lactamase detection was performed by double-disc synergism test. The virulence genes profile performed was *iroN*, *iucD*, *irp-2*, *rmpA*, *magA*, *K2*, *kfu*, *uge*, *K1*, *kpn*, *mrkD*, *fimH*, *cc258*, and *allS*. ESBL-producing *K. pneumoniae* strains were subjected to whole genome sequencing on Illumina NextSeq platform using paired-end library. MLST, resistance genes, plasmids, pMLST were determined using online tools of Center for Genomic Epidemiology. Among the 19 strains of *Klebsiella* spp., 16 (16/19) were identified as *K. pneumoniae*, and three (3/19) as *K. oxytoca*. The antimicrobial susceptibility profile demonstrated high resistance to ampicillin (89.5%), multiple resistance (at three or more antimicrobial categories) was detected in 31.6% (6/19) of the strains, and the standard of resistance to ampicillin, sulfonamide, and gentamicin was the most prevalent (50%, 3/6). The virulence profile demonstrated a high prevalence of *fimH* (94.7%), *kpn* (89.4%), *uge* (84.2%) and *irp-2* (78.9%). Three strains of *K. pneumoniae* were positive for extended-spectrum beta-lactamase production. These strains were classified in sequence types (STs) ST15, ST147 and ST307. These three clonal groups represent the main responders for outbreaks of *K. pneumoniae* nosocomial infections worldwide. However, this is the first account of these clones as causing disease in birds. These data indicate the occurrence of *K. pneumoniae* producing CTX-M-15 and CTX-M-8 in captive parrots and confirm the zoonotic and anthroozoonotic potential of the agent, highlighting the clinical relevance for humans and animals.

Keywords: Psittacines. *Klebsiella* spp. Microbiology. Bacterial resistance. ESBL.