Multidrug-resistant Escherichia coli and Klebsiella spp. with virulence determinants in healthy household dogs: a One Health perspective

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Antimicrobial resistance is a critical public health issue affecting humans, animals, and the environment. Healthy household dogs may serve as reservoirs of resistant bacteria and resistance genes. This study investigated antimicrobial-resistant Gram-negative bacilli (GNB) in rectal swabs from 121 clinically healthy dogs (106 adults and 15 puppies) from households in cities in the interior of São Paulo and Minas Gerais states, Brazil. None of the dogs had received antimicrobial treatment within the previous 30 days. The population was diverse, predominantly mixed-breed (55%), among 19 other breeds. Bacterial isolates were selected using MacConkey agar supplemented with 2 µg/mL of cefotaxime. Identification was performed using MALDI-TOF MS and the *Klebsiella* MALDI TypeR platform. Antimicrobial susceptibility testing was performed by disk diffusion method with 14 antibiotics. ESBL production was detected using the double-disk synergy test, and PCR was conducted to identify 42 genes encoding resistance genes to beta-lactamases, quinolones, fluoroquinolones, aminoglycosides, and polymyxins, as well as virulence determinants (29 for *Escherichia coli* and 20 for *Klebsiella* spp.). A total of 68 GNB isolates were recovered, of which 43/68; 63% were *Enterobacterales*, predominantly *E. coli* (23/43) and *Klebsiella* spp. (6/43). Multidrug resistance (MDR) and ESBL production were highly prevalent among both species, with MDR detected in 18/23 (78%) *E. coli* and 5/6 (83%) *Klebsiella* spp. isolates, while the ESBL phenotype was identified in 12/18 (67%) and 4/5 (80%) of MDR isolates, respectively. Overall, MDR *E. coli* and *Klebsiella* spp. exhibited broad and distinct antimicrobial resistance profiles. All *E. coli* isolates were confirmed to be resistant to cefotaxime, with resistance to tetracycline (83%), quinolones (67% to enrofloxacin and 72% to ciprofloxacin), fluoroquinolones (61% to enrofloxacin and 72% to ciprofloxacin), beta-lactams and trimethoprim/sulfamethoxazole (50–56%), and aminoglycosides (17%). In contrast, *Klebsiella* spp. confirmed resistance to cefotaxime, with resistance to tetracycline and trimethoprim/sulfamethoxazole, with 80% resistance rates across multiple classes, including beta-lactams and fluoroquinolones, and detection of carbapenem resistance (20%). Genotypically, $bla_{\text{CTX-M group 1}}$ predominated in both species (61% in *E. coli* and 80% in *Klebsiella*), followed by *aac(6')-Ib* (33% and 20%, respectively), while $bla_{\text{CMY-like}}$ and $bla_{\text{SHV-like}}$ were detected mainly in *E. coli*. Notably, a bla_{KPC} was identified in a *K. pneumoniae* isolate, highlighting the emergence of clinically critical resistance. MDR *E. coli* isolates frequently harbored virulence-associated genes, particularly those related to adhesion (*fimH*), iron acquisition (*fyuA*, *iutA*), and

serum resistance (*traT*). In addition, some isolates carried genes associated with extraintestinal pathogenicity, including pap operon genes, *hlyA*, and *kpsMTII*. MDR *Klebsiella* spp. isolates carried virulence-associated genes mainly related to adhesion and colonization (*fimH*, *mrkD-1*), capsule and lipopolysaccharide biosynthesis (*wabG*, *ugE*), and iron acquisition systems (*entB*, *kfuBC, fecIRA*), supporting their ability to persist in the host and cause opportunistic infections. The presence of multidrug resistance and virulence determinants highlights the potential of clinically relevant *Enterobacterales* to act as opportunistic pathogens, reinforcing their importance in One Health surveillance.

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