

Amphibian Species as Sentinels for Monitoring Antimicrobial Resistance Trends in the Environment

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Antimicrobial resistance (AMR) is considered one of the greatest threats to global health due to its impact on public, veterinary and environmental health. Wild animals, although not directly exposed to clinically relevant antibiotics, can be colonized by resistant bacteria due to changes in their ecosystems by anthropogenic activities. The flow of bacteria and resistance genes is probably structured by ecology, species that share similar niches drawing from similar gene pools, where colonization and gene transfer occur. Therefore, antibiotic resistant bacteria and wild animals can be used as bioindicators and sentinels, respectively. The aim of this study was to investigate the presence of antibiotic-resistant Gram-negative bacteria in cutaneous microbiota of frogs, from the Mata Atlântica biome in São Paulo, Brazil. Skin swabs of *Phyllomedusa distincta* ($n=20$), *Rhinella* spp. ($n=5$); *Scinax* spp. ($n=3$) and *Physalaemus* spp. ($n=2$) were collected and inoculated on MacConkey agar plates supplemented with cefotaxime (2 µg/ml). Bacterial isolates were identified by 16S rRNA, MALDI-TOF/MS and Vitek®2, and their resistance phenotypes were determined by disk diffusion method, using CLSI and EUCAST breakpoints. From one-hundred-fifteen isolates recovered, *Enterobacter cloacae* ($n=2$), *E. bugandensis* ($n=1$), *Pseudomonas fulva* ($n=2$), *Stenotrophomonas maltophilia* ($n=4$), *Leclercia adecarboxylata* ($n=1$), *Citrobacter freundii* ($n=1$) and *P. putida* ($n=1$) displayed a multidrug-resistant profile to clinically relevant antibiotics, including broad-spectrum cephalosporins (*i.e.*, cefotaxime, ceftazidime, and ceftriaxone), carbapenems (meropenem, ertapenem), fluoroquinolones (ciprofloxacin), trimethoprim-sulfamethoxazole and/or tetracyclines. Additionally, a *P. japonica* isolated from the aquatic environment of *Rhinella* spp. exhibited resistance to meropenem and ceftazidime. To our knowledge, this study provides the first insight into the occurrence of multidrug-resistant bacteria in wild frogs, supporting a potential as sentinel for monitoring antimicrobial resistance trends in the environment. Genomic analyzes allows elucidate the resistome (antibiotic, biocides, agrotoxics, and heavy metals), in order to support this sentinel potential.

Keywords: Antimicrobial resistance, wild animals, amphibian, carbapenems, WGS.

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