Antibiotic resistance and virulence factors in the marine environment:
Cetaceans as sentinels

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ABSTRACT

Antibiotic resistance has been a major threat to human and animal health because pathogenic microorganisms are becoming resistant to the most prescribed antibiotic treatments, leading to prolonged illness and greater risk of death. However, the detection of antimicrobial resistance in nonclinical settings has been receiving little focus in research. Of special interest are aquatic environments because, water, which provides easy access to dissolved nutrients, as well as protection from desiccation and ultraviolet light, is an ideal medium for microorganisms. Both freshwater and marine systems act as a sink for bacteria that enter aquatic systems via treated and untreated sewage, hospital waste, agricultural run-off, and other anthropogenic sources, all of which can harbor different levels of antibiotic resistance and virulence. Reports of antimicrobial resistance in marine animals are fewer compared to terrestrial animals, but these studies are significant in relation to marine mammal stranding and rehabilitation activities.

This study generally aimed to determine the antibiotic resistance patterns and detect virulence factors of bacteria isolated from select cetaceans that stranded in the Philippines in 2018. Specifically, this study aimed to (1) confirm the phenotypic and molecular identifications of isolated Gram-negative bacteria ; (2) test the susceptibility of bacterial isolates to 16 antibiotics representing seven antibiotic classes; (3) detect target virulence genes in selected bacterial groups; (4) find significant association between stranding event parameters (e.g., age class, sex, species, stranding location, etc.) and antibiotic resistance patterns as well as presence of virulence factors in bacterial isolates. Bacteria that were isolated from the stranded cetaceans were identified phenotypically with the use of Gram-staining, morphological description, and biochemical reactions with the aid of VITEK® 2 Identification System. Molecular identification was confirmed through 16S rRNA gene amplification and sequencing. Only the genera with high number of isolates were subjected to antibiotic susceptibility profiling and virulence factors detection. Testing of sensitivity to different antibiotics was done through automated antibacterial susceptibility assay (VITEK® 2).

Associations between patterns of virulence and stranding event parameters were determined using Fisher's exact test. Out of the 76 bacterial isolates subjected to assays, 39.47% exhibited acquired resistances against penicillins (5.26%), cephalosporins (32.89%), carbapenems (1.32%), polymyxins (6.58%) and sulfonamides (7.89%). In addition, 63.16% 64.47% were found to have virulence genes present: *fimH*, *stx1*, and *stx2* in *Escherichia* (96.97%), *ascV*, *entB*, and *mrkD* in *Enterobacter* (37.5%), and *clbA*, *wcaG*, and *ybtS* in *Klebsiella* (42.11%). No significant association was found between the antibiotic resistance and virulence profile, between the biological source, cetacean species, and cetacean age class. These results show the importance of surveillance of antibiotic resistance and virulence profile on stranded cetaceans as a tool for continuous monitoring ocean health.

Keywords: cetaceans, antibiotic resistance, virulence, stranding