HEAVY METAL RESISTANCE IN BACTERIA ISOLATED FROM HUMBOLDT PENGUIN (*SPHENISCUS HUMBOLDTI*) EXCRETA

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Introduction Heavy metal pollution is one of the most serious environmental problems caused by man (Blais et al., 2007). Due to mining and industrial development in Chile, an increasing level of metals in coastal ecosystems has been noted (Salamanca et al., 2004). In these areas several colonies of Humboldt penguins are concentrated, which can be affected by these anthropogenic activities (Celis et al., 2014). Heavy metals are incorporated into the animal through diet, and most part of them are excreted on land (Celis et al., 2014). Heavy metals can alter the microbial flora that live on the ground (Montuelle et al., 1994). Thus, pollution of coastal ecosystems can have impacts on native bacteria by the emergence of resistant strains. The consequences can be worrisome for the environment and health (Nies, 2003). Globally, 70% of emerging infectious diseases originated in wildlife (Jones et al., 2008). Contamination by heavy metals on microbial flora has not been fully studied in fauna of coastal areas of Chile (Moraga et al., 2003). Exposure to heavy metals could be influencing the susceptibility of gastrointestinal bacteria of this species. Furthermore, studies have shown that metal contamination could have an important role in the proliferation of antibiotic resistance (Rose et al., 2009). Little is known if heavy metal pollution has an real impact on the microbial flora of these birds in relation to habitats. In this study, the aim was (1) to determine bacterial resistance to metals such as Cd, As, Pb, Cu and Zn in excreta of Humboldt penguins that nest in three important areas of the Chilean coast, and (2) investigate whether metals have any effect on bacterial resistance to some antibiotics.

Methods Ten fresh excreta samples of Humboldt penguins were collected from each nesting sites. Samples were stored at ~ 4 ° C until their arrival to the laboratory. Sampling was conducted between December 2011 and January 2012. The locations studied were: Pan de Azucar Island (26º09'S, 70º40'O), Chañaral Island (29º01'S, 71º34'O) and Cachagua Island (32º35'S, 71º27'O).

The microbiological trials were performed at the Faculty of Veterinary Science, University of Concepcion. Samples were grown in buffered peptone broth (37°C x 24 h). Then, samples were transferred to three selective media for the isolation of bacterial colonies which were incubated again. Bacterial identification from isolated colonies, was based on studies of microscopic morphology, Gram staining and biochemical properties. A standardized system for bacterial identification was used for the characterization of the identified species (API).

The bacterial resistance to heavy metals were tested with 3200, 1600, 800, 400 and 200 µg / mL for As, Cu and Pb; for Zn were 1600, 800, 400, 200 and 100 µg / mL, whereas for the Cd levels tested were 400, 200, 100, 50 and 25 µg / mL (Mondaca et al., 1993). Bacterial resistance was determined by the minimum inhibitory concentration (MIC) for these metals (Anisimova et al., 1993).

All strains isolated were also tested for resistance to a wide range of antibiotics oxytetracycline (30 mg / mL), penicillin (10 µg / mL), gentamicin (10 µg / mL), florfenicol (30 µg / mL) amoxicillin (20 mg / mL),

sulfatrimet (25 µg / mL) and cefapirin (30 µg / mL). The concentration of the antibiotic used was greater than the maximum inhibitory concentration listed on performance standards CLSI (2014), except for florfenicol. The results were recorded as sensitive or resistant.

Results Bacterial resistance to heavy metals varied from penguin colonies inhabiting the studied locations, with greater resistance to As, Cu and Pb in strains isolated from feces of Cachagua Island, being this site which exhibited the highest concentrations reported for these elements. In general, all strain isolated showed resistance to metal Zn. The most toxic metal was Cd, followed by Cu. All Gram (+) and Gram (-) isolated were susceptible to oxytetracycline, gentamicin, florfenicol, amoxicillin and cephapirin. All Gram (-) isolated were resistant to penicillin and some to sulfatrim. There was no bacterial resistance to metals associated with antibiotic resistance.

Conclusion The resistant strains to heavy metals found in this study could affect organic and inorganic environments cycles in northern and central coast of Chile. Although bacterial resistance to metals in the areas studied was not associated with resistance to antibiotics, further research is required. The habitats occupied by the Humboldt penguin had different concentrations of heavy metals. This is interesting because it allows locating the areas where human activities impact mostly on these species of seabirds. All this would be a great contribution to assess the degree of contamination due to anthropogenic activity and could be affecting the wildlife of the Chilean coast and human health, which will support making appropriate decisions to reduce pollutants in the environment.

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