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# Antimicrobial Resistance Levels among Gram-negative Bacteria from Peruvian Boobies (*Sula variegata*) in Northern Peru

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ABSTRACT: The presence of antimicrobial-resistant bacteria in feces of 42 Peruvian Boobies (*Sula variegata*) from a Northern Peru island was evaluated using MicroScan and disk diffusion. Fourteen microorganisms were recovered, including three *Pseudomonas* spp. resistant to one antibiotic each, and four multiresistant *Escherichia coli*. Antimicrobial-resistant bacteria are reported in *S. variegata*.

The high levels of use of antimicrobial agents have resulted in an increasing description of antimicrobial-resistant bacteria (ARB) or antimicrobial resistance genes (ARGs) in all environments, including wild animal microbiomes, which in turn can become stable or unstable reservoirs and possible spreaders for ARB (Dolejska and Literak 2019).

In Peru, access to antibiotics is uncontrolled and self-medication is a common event (Zavala-Flores and Salcedo-Matienzo 2020), but data regarding resistance to antimicrobial agents are fragmented, partially outdated, and mainly focused on human health (Ochoa et al. 2009; Palma et al. 2017). No study has been performed focusing on the presence of ARG in guano-producing birds.

We aimed to detect ARB in cloacal swabs of Peruvian Boobies (*Sula variegata*) from Guañape Norte island (8°32′41″S, 78°57′49″W), a rocky and barren island within a National Reserve (Reserva Nacional Sistema de Islas, Islotes y Puntas Guaneras), located 16 km offshore of the northern coast of Peru.

Peruvian Boobies live in permanent colonies in the island, together with other seabirds such as Guanays (*Phalacrocorax bougainvilliorum*) and Humboldt Penguins (*Spheniscus humboldti*), and sea mammals such as the South American sea lion (*Otaria flavescens*). Unlike other scavenging seabirds, *S. variegata* do not feed on fish discards or offal but rather primarily on Peruvian anchovies (*Engraulis ringens*), both in coastal or continental shelf break and slope (100–2,500-m deep) waters (Goya Sueyoshi 2000; Zavalaga et al. 2010).

Two rangers live year-round on the island and access for researchers is limited to a few short ad-hoc visits a year. Guano harvesting (with hundreds of workers living permanently on the island for months) is carried out every 4–6 yr (García et al. 2016); the last guano campaign occurred during 2014. On this island, based on their feeding habits and controlled human intervention in their breeding sites, *S. variegata* are good candidates for monitoring the presence of ARB.

Forty-two chick-rearing *S. variegata* adults were captured during November 2019 as previously described (Zavalaga et al. 2010). Each cloacal swab was collected on Cary Blair transport media and stored at 4 C until transferred to the laboratory (within 12–19 d).

We used standard bacterial culture methods (Murray et al. 2007). Colonies suspected of being *Escherichia coli*, *Salmonella enterica*, *Pseudomonas* spp., *Acinetobacter* spp., and *Klebsiella* spp. were collected, isolated, and identified using the MicroScan automated system (Siemens Medical Solutions Diagnostics, Camberley, UK), which is based on microtitre panels containing dried metabolites, allowing identification of microorganisms through biochemical reactivity.

We determined susceptibility to aminoglycosides (amikacin, gentamicin), β-lactam (ampicillin), β-lactam plus inhibitors (piperacillin plus tazobactam), carbapenems (imipenem), cephalosporins (ceftriaxone, cefotaxime, cefoxitin), monobactams (aztreonam), folate inhibitors (cotrimoxazole), nitrofurans (nitro-

TABLE 1. Antimicrobial resistance levels among bacterial isolates from fecal samples from adult Peruvian Boobies (*Sula variegata*) from a northern Peru island, collected November 2019.[a]

| | N | AMP N (%) | FOX N (%) | AZM N (%) | NAL N (%) | CIP N (%) | SXT N (%) | TET N (%) | GEN N (%) |
|---|---|---|---|---|---|---|---|---|---|
| *Acinetobacter* sp. | 1 | 0 (0.0) | 0 (0.0) | NT | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| *Escherichia coli* | 6 | 5 (83.3) | 0 (0.0) | 3 (50.0) | 2 (33.3) | 2 (33.3) | 3 (50.0) | 4 (66.7) | 1 (16.7) |
| *Pseudomonas* sp. | 7 | NT | 1 (14.3)[b] | NT | 0 (0.0) | 1 (14.3) | 1 (14.3)[b] | 0 (0.0) | 0 (0.0) |

[a] AMP = ampicillin; FOX = cefoxitin; AZM = azithromycin; NAL = nalidixic acid; CIP = ciprofloxacin; SXT = cotrimoxazole; TET = tetracycline; GEN = gentamicin; NT = not tested (antibiotics were tested according to bacterial species standards). No isolate was resistant to any of the remaining antibiotics analyzed in the study.

[b] *Pseudomonas* sp. with cefoxitin and cotrimoxazole halo diameter <10 mm were considered as resistant, because no breakpoint was available.

furantoin), macrolides (azithromycin), quinolones (nalidixic acid, ciprofloxacin, levofloxacin), and tetracyclines (tetracycline) by disk diffusion according to European Committee on Antimicrobial Susceptibility Testing guidelines (2020). Susceptibility of *E. coli* to nalidixic acid and tetracycline was established following Clinical and Laboratory Standards Institute guidelines (2020). We used the *E. coli* azithromycin-breakpoint (15 mm) proposed by Ochoa et al. (2009). Multidrug resistance was defined as resistance to at least one antibiotic belonging to a minimum of three different antibiotic families (Ruiz-Roldán et al. 2018). We tested for the presence of extended spectrum β-lactamases and inducible pAmpC as described elsewhere (Palma et al. 2017; Ruiz-Roldán et al. 2018), but none were detected.

We recovered 14 colonies (14/42; 33.3%): seven *Pseudomonas* spp., six *E. coli*, and one *Acinetobacter* sp. The *Acinetobacter* sp. was pansusceptible. Three *Pseudomonas* spp. were resistant to one antimicrobial each (ciprofloxacin, cefoxitin, and cotrimoxazole, respectively) (Tables 1, 2). The *E. coli* isolates were susceptible to antimicrobial agents such as cephalosporins or carbapenems, but showed high levels of resistance to older antimicrobial agents such as ampicillin (83.3%), tetracycline (66.7%), cotrimoxazole (50.0%), and gentamicin (16.7%), as well to a few more recent agents, such as azithromycin (50.0%), and to fully synthetic antimicrobials, nalidixic acid and ciprofloxacin (33.3%). We found four *E. coli* isolates (66.7%) to be multidrug resistance; two of these were resistant to five unrelated antibiotic families (Tables 1, 2).

TABLE 2. Isolates of bacteria from fecal samples from adult Peruvian Boobies (*Sula variegata*) from a northern Peru island, collected November 2019, showing resistance to at least one antibiotic tested.

| Isolate | Species | Resistance pattern[a] | Multidrug resistance |
|---|---|---|---|
| N10 | *Escherichia coli* | AMP, NAL, CIP, SXT, TET, GEN | Yes |
| N15 | *Escherichia coli* | AMP, NAL, CIP, SXT, TET, AZM | Yes |
| N18 | *Escherichia coli* | AMP, SXT, TET | Yes |
| N29 | *Escherichia coli* | AZM | No |
| N30 | *Escherichia coli* | AMP | No |
| N38 | *Escherichia coli* | AMP, TET, AZM | Yes |
| N25 | *Pseudomonas* sp. | FOX[b] | No |
| N39 | *Pseudomonas* sp. | SXT[b] | No |
| N41 | *Pseudomonas* sp. | CIP | No |

[a] AMP = ampicillin; NAL = nalidixic acid; CIP = ciprofloxacin; SXT = cotrimoxazole; TET = tetracycline; GEN = gentamicin; AZM = azithromycin; FOX = cefoxitin.

[b] Disk diameter halo = 0 mm.

Because of frequent use of antibiotics, human pathogenic and commensal *E. coli* often exhibit antimicrobial resistance (AMR) (Ochoa et al. 2009). The AMR *E. coli* isolated could reflect human influence during guano or seabird surveys. Other origins, such as the arrival to the island through water current, or other birds feeding in landfills, need to be considered. Conversely, the detected *Acinetobacter* and *Pseudomonas* spp. might reflect low direct antibiotic pressure in *S. variegata* feeding areas.

Previous reports have showed presence of ARB in seabirds from remote areas. A few ARBs, including two extended spectrum β-lactamases carrier bacteria, were recovered from seabirds, including Masked Booby (*Sula dactylatra*) from Easter Island. Of note, Easter Island had approximately 4,500 stable inhabitants, and a similar number of daily visitors in touristic season (Ardiles-Villegas et al. 2011). Likewise, Sjölund et al. (2008) report the presence of AMR *E. coli* (mostly showing resistance to antimicrobials such as ampicillin or chloramphenicol) in feces of Artic seabirds; fisherman, settlers, scientists, and migratory birds are all proposed as possible explanations. The risk of the transmission of these AMR *E. coli*, or the ARGs, to humans through the food chain via the use of guano in agriculture must be considered. A recent study by Esperón et al. (2020) analyzing poultry manure showed that after 10 wk, while several ARGs, such as $bla_{TEM}$, *qnr*S, or *tet*(A) tended to decrease, others such as *tet*(Y), *aad*A, or *sul*I tended to increase. The main limitation is the time elapsed between sample collection and culture. According to the nature of the sampling on an island with only ad hoc communication with the mainland, the samples remained stored at 4 C in Cary Blair transport medium for up to 19 d, which might have impacted the viability of several bacterial species (Dan et al. 1989). Nevertheless, our data clearly demonstrate the presence of ARB in cloacal swabs of *S. variegata* from Northern Peru. Further work is needed to analyze the origin and transmission risk of these bacteria or their encoded ARGs.

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