Science of the Total Environment 777 (2021) 146004

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

Upraising a silent pollution: Antibiotic resistance at coastal environments and transference to long-distance migratory shorebirds

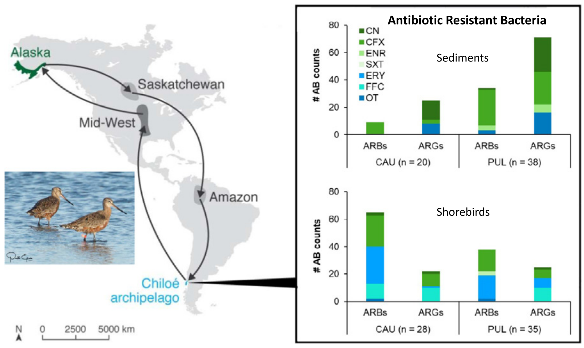
Juan G. Navedo [a,b,*], Valeria Araya [a], Claudio Verdugo [c]

[a] *Bird Ecology Lab, Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Chile*
[b] *Estación Experimental Quempillén (Chiloé), Facultad de Ciencias, Universidad Austral de Chile, Chile*
[c] *Ecology and Evolution of Infectious Diseases Lab, Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Chile*

HIGHLIGHTS

- ARB and ARGs are widespread in sediments and shorebirds in Chiloé, Pacific Patagonia.
- Antibiotics used in salmon aquaculture boost the occurrence of multi-resistant ARB.
- Migratory shorebirds are reservoirs and potential spreaders of ARB and ARGs.
- The antibiotic footprint is clearly detected even at a sparsely-populated area.
- Limiting antibiotic inputs to protected areas seems urgent under One-health approach.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 November 2020
Received in revised form 14 February 2021
Accepted 16 February 2021
Available online 27 February 2021

Editor: Yolanda Picó

Keywords:
Biodiversity conservation
Ecotoxicology
One-health
Patagonia
Salmon aquaculture
Wetlands

ABSTRACT

Large amounts of antibiotics from different sources have been released into coastal environments, especially in high human-populated areas, but comprehensive studies of antibiotic footprint in wildlife are scarce. Here we assess occurrence of antibiotic resistant bacteria (ARB) and antibiotic resistance gene (ARG) both in sediments and gut microbiota of a long-distance migratory shorebird species in two coastal wetlands at a sparsely-populated area in Pacific Patagonian coasts with contrasting potential antibiotic sources, especially from aquaculture. We found 62% of sediment samples showing ARB, and ARGs similarly occurring in sediments at both bays. However multi-resistant ARB were found only at sediments in the bay surrounding aquaculture operations. An 87% of cloacal bird samples showed at least one ARB, with 63% being multi-resistant and some of them with a high potential pathogenicity. ARGs were present in 46% of the samples from birds, with similar multi-resistant frequencies among bays. Besides specific differences mainly associated to antibiotics used in salmon aquaculture that boosted ARB in sediments, ARB and ARGs occurrence was overall similar at two bays with contrasting main human activities, in spite of being a comparatively low human-populated area. Therefore, our results reinforce the idea that the antibiotic footprint may be widespread at a global scale and can extend beyond the geographical influence of antibiotic sources, especially at coastal environments where migratory shorebirds act both as reservoirs and potential spreaders of antibiotic resistance.

1. Introduction

Antibiotics are among the most widely administered drugs used for human health and growth management in agriculture, livestock and aquaculture worldwide (Sarmah et al., 2006). The increased and

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* Corresponding author at: Bird Ecology Lab, Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Chile.
E-mail address: jgnavedo@uach.cl (J.G. Navedo).

https://doi.org/10.1016/j.scitotenv.2021.146004

widespread use of sub-therapeutic doses of antibiotics, along with effluents from sewage treatment plants to rivers, inlets and estuaries, have been responsible for large amounts released into the environment (Ben et al., 2019). Therefore, coastal wetlands can be ideal scenarios for the development of antibiotic resistant bacteria (hereafter ARB) (Taylor et al., 2011), one of the major threats to human well-being (Cunningham et al., 2017). For this reason, an encouragement to prevent antimicrobial overuse and its detrimental effects on the health of wildlife, human beings, and the aquatic ecosystem had been globally adopted (i.e. 'One health' notion) (Cabello et al., 2016). Specifically framed by 'One health' are the interactions of human sources of pollution with animals and the environment as potentials reservoirs or spreaders of such pollutants (Cabello et al., 2016).

The heavy use of prophylactic antibiotics in aquaculture can be particularly relevant by creating a selective pressure in areas of the marine environment far from its initial site of use (Cabello et al., 2013), assisting in ARB selection in coastal marine sediments (Tomova et al., 2015). A recent study experimentally demonstrated that commercial (i.e. non-medicated) fishmeal induced antibiotic resistance gene (hereafter ARG) propagation in bacteria within aquaculture sediments (Han et al., 2017). ARGs from different sources can be found widespread at coastal sediments (e.g. 4000 km of coasts in China; Zhu et al., 2017); however, comprehensive studies accounting for ARBs and ARGs in the aquatic environment and transference to associated wildlife are scarce (Marcelino et al., 2019).

In general, ARB can carry ARGs that can be exchanged among bacteria via horizontal gene transfer (Allen et al., 2010). Therefore, the presence of ARGs implies that resistance information is being exchanged between bacteria, potentially leading to the transfer of these resistance properties to pathogens and increasing the number of bacteria carrying the resistance gene (Cabello, 2006). When transferred to pathogenic bacteria, antibiotic resistance elements represent an important problem not only for human health (Baker et al., 2018), but also for wildlife. Migratory waterbirds can act as vectors for seeds, invertebrates or microbes (Bauer and Hoye, 2004), and their exposure to ARB may promote the dispersion from the digestive tract of reservoir individuals to distant locations (Allen et al., 2010), and may also affect the birds' own microbiota with consequential effects (Morgun et al., 2015) that remain unknown. If acquired by waterbirds, ARB and ARGs may be thus spread during migration to staging and breeding areas located several thousands of kilometers apart from antibiotic sources (Hernández et al., 2013). Assessing links between ARB and ARGs in sediments and wildlife are therefore key for our understanding of the impact of antibiotic footprint on global biodiversity.

In South America, a very scarce development of sewage treatment systems, weak environmental laws and virtually absent enforcement of compliance, have led to effluents from housing and small local hospitals being released as untreated waste water directly to coastal environments (Quiñones et al., 2019). In addition, the Chilean salmon industry has intensively used antibiotics in the last decades to prevent and control stock mortalities (Niklitschek et al., 2013). For many years, oxolinic acid, amoxicillin, erythromycin, flumequine, florfenicol and oxytetracycline were used at some of the highest rates among the salmon production countries (Miranda et al., 2018). The total amount of antibiotics administered annually from 2012 up to 2017 ranged between 383 and 577 tons (SERNAPESCA, 2017), with florfenicol and oxytetracycline being the most common (Miranda et al., 2018). The Chiloé Archipelago (41–43°S, Los Lagos Region, Chile) is currently a major area for salmon aquaculture at a global scale and, as such, a 30.4% of the total amount of antibiotics reported in Chile is used in the surrounding coasts of Chiloé (SERNAPESCA, 2017). In fact, some studies demonstrated the presence of ARB and ARGs in sediments around Chiloé against the most used antibiotics in salmon aquaculture (Buschmann et al., 2012; Aedo et al., 2014), and even scaled up to local hospitals (Tomova et al., 2015).

In parallel, some long-distance migratory Nearctic shorebird populations fly annually from relatively unpolluted breeding grounds in the Arctic to the southern cone of South America, mainly to two big archipelagos, Tierra del Fuego and Chiloé, where they spent the non-breeding season (Andres et al., 2009; Navedo and Gutiérrez, 2019). Although Patagonian bays remained sparsely inhabited and unindustrialized until contemporary times, global change has driven severe modifications including antibiotic input sources (Barton and Floysand, 2010). However, whether migratory waterbirds inhabiting this important conservation area are carrying or not ARB and ARGs is currently unknown.

Here we assess the presence of ARB and ARGs in the environment against antibiotics from different sources and potential coalescence to gut microbiota of wildlife. We select as a model a long-distance shorebird belonging to worldwide-threatened Numeniini tribe (Pearce-Higgins et al., 2017) that seasonally spends half of its annual cycle inhabiting intertidal areas. Whether or not these animals currently harbor and spread ARB and ARGs is of global concern for human well-being since they link ecosystem processes between distant areas via trophic and transport effects (Bauer and Hoye, 2004).

2. Methods

2.1. Study area and fieldwork

We focused our research in two coastal wetlands heavily used by migratory shorebirds within the Chiloé Archipelago but differing in potential antibiotic sources. Pullao bay is within an important area of salmon aquaculture with no major human development at the shorefront. In contrast, Caulín bay is located 70 km northwards, where a small community inhabit there and a small health facility is placed in the shorefront. The closest salmon facilities are located at a distance of c. 20 km of Caulín bay (Fig. 1).

The Hudsonian godwit *Limosa haemastica* (Linnaeus, 1758) (hereafter godwits) is a migrant Nearctic species with 99% of its Alaskan population spending the non-breeding season (Oct-Mar) in Chiloé (Senner et al., 2014) (Fig. 1a). Godwits made long distance non-stop migratory bouts, typically staging in two inland wetland areas, one in Canadian prairies and the other in high-amazon floodplains during southward migration (Senner et al., 2014). Once in Chiloé godwits forage mainly on benthic worms and exclusively at intertidal areas (Navedo et al., 2019). Despite the short distance (<70 km), godwits from both bays do not mix extensively (J.G. Navedo unpubl. data from GPS-tagged individuals) and show a very high phylopatry (Senner et al., 2014). Godwits at these two contrasting bays in Chiloé provide, therefore, an excellent model to test the potential transference of antibiotics present in coastal sediments to wildlife.

Sediment samples were obtained from both bays (Caulín $n = 20$, Pullao $n = 38$) during low tide on consecutive days in October 2017. Collections were conducted every 100 m by walking along a transect parallel to the shoreline of each bay. Sediments were collected with sterilized PVC cores by removing the first 3 cm, and immediately stored with sterilized plastic bags at 4 °C until laboratory processing. Godwits were captured using cannon-nets during early December 2017 and March 2018 (details in Gherardi-Fuentes et al., 2020). Individual cloacal samples (Caulín $n = 28$, Pullao $n = 35$) were obtained using sterile swabs and immediately placed into Amies transport medium at 4 °C for transport to the laboratory.

2.2. Laboratory analyses

Bacterial cultures from sediments and cloacal samples to target host bacteria taxa were incubated in brain heart infusion media (BD Diagnostics, MD, USA), and then plated in 7% XLD blood agar plates, specifically containing 5 $g \cdot l^{-1}$ of NaCl (Oxoid Ltd., UK). The antibiotic susceptibility was tested following by Kirby-Bauer disk diffusion assay in 10^{8-9} CFU/ml of bacterial suspension in Mueller-Hinton media (Oxoid Ltd., UK) following the Clinical and Laboratory Standards

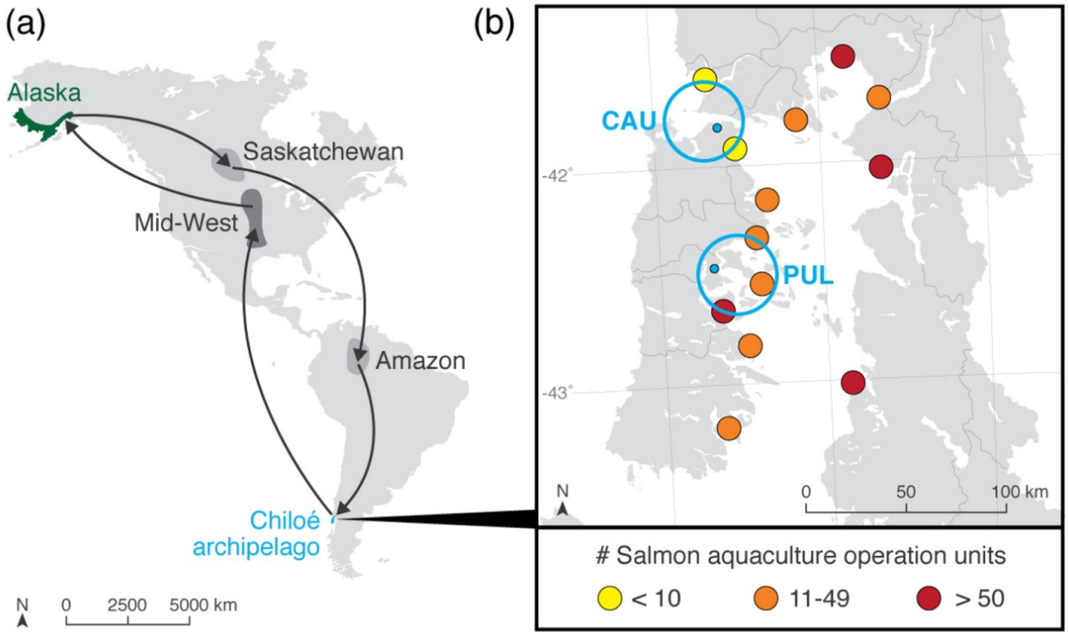

Fig. 1. a) Range map and main staging areas used by Hudsonian godwits breeding in Alaska throughout the annual cycle (composed from Senner et al., 2014). b) Geographical location of the two studied bays (blue points: CAU and PUL) within Chiloé Archipelago and main home-range of Hudsonian godwits around each studied bay (J.G. Navedo unpubl data from GPS-tagged individuals), indicating current salmon aquaculture operation units at each administrative area ('comunas') within Chiloé inner sea (official data from SUBPESCA, Gobierno de Chile, 2017). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Institute (CSLI) guidelines (CSLI 2008). Colonies were tested against eight antibacterial substances (Oxoid Ltd., UK), three from salmon aquaculture source, oxytetracyclin (OXT; 30 μg), florfenicol (FFC; 30 μg) and erythromycin (ERY; 15 μg); and five from medical/veterinarian sources commonly used in this area, gentamicin (CN; 10 μg), enrofloxacin (ENR; 5 μg), sulphamethoxazole/trimethoprim (SXT; 25 μg), cefoperazone (CFP; 75 μg) and cefuroxime (CXM; 30 μg). *Escherichia coli* ATCC 25922 was used as a control. The diameter of inhibition zones was measured and classified as susceptible, intermediate resistant or resistant, according to CLSI standard measurements for each antibiotic and bacteria genera when available; otherwise, breakpoints were those for Enterobacteriaceae. Because those standards were developed for clinical conditions and model bacteria, intermediate and resistant phenotypes found in natural environment and wildlife were treated as ARBs under a 'One-health' approach.

In order to identify the bacteria from each isolates, DNA was extracted using a commercial kit following manufacturer's instructions (E.Z.N.A.® Bacterial DNA Kit, Omega GA, USA) for the amplification of the 16S rRNA gene (~1500 bp) using universal primers (Montagner et al., 2010) and, then, sequenced in both directions. Bacterial identification was performed by sequence identity against the GENBANK database.

2.3. Detection of antibiotic resistance genes

Using the DNA extraction method mentioned above, the presence of 21 ARGs from a variety of molecular mechanisms and all eight antibiotics were determined (Supplementary material; Tables S1, S2): Oxytetracyclin resistance genes (tetA, tetM (Aarestrup et al., 2000; Guardabassi et al., 2000)), gentamicin resistance genes (aac(6′)-Ie-aph(2″)-Ia, ant(4′)-Ia, aph(2″)-Ib, aph(2″)-Ic, aph(2″)-Id and aph(3″)-IIIa (Vakulenko et al., 2003)); florfenicol resistance gene (floR (Keyes et al., 2000)); enrofloxacin (qnrA, qnrB, and qnrS (Robicsek et al., 2006)); sulphamethoxazole/trimethoprim mobile element (dfrA12 (Grape et al., 2007)), cefoperazone and cefuroxime resistant genes (blaTEM (Colom et al., 2003) and blaFOX (Coudron et al., 2003)); and erythromycin resistant genes (ermA, ermB, ermC (Martineau et al., 2000), mefA, and mefF (Woodbury et al., 2008)). Amplicons of the expected sizes from some (three when available) selected samples were excised from gel and sequenced to check correct targeting.

2.4. Statistical analyses

Phenotypes of samples of sediments and birds obtained from the inhibition halo were scored as resistant, intermediate, or susceptible according to CSLI guidelines and used for further analyses. Differences on the frequency of ARB among bays were calculated using Chi-squares and Fisher's Exact test for count data in contingency tables. A Yate's continuity correction was used in those with expected frequency of less than 5. Frequencies of ARGs were compared among bays using Chi-squares in contingency tables with a Yate's continuity correction when necessary. Confidence intervals of the proportions were calculated using Clopper–Pearson exact method. ARB and ARGs were then grouped by aquaculture and medical/veterinarian sources to explore for potential differences between bays. All analyses were performed in R Studio platform and p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Sediments

A total of 62% (36/58) of the samples from sediments showed ARB against at least one antibiotic, and 94% of these (34/36) were against CXM (Fig. 2a). All isolates showed susceptibility to SXT and FFC. Pullao bay (71%, CI: 54.1–84.5%) showed significantly more ARB to at least one antibiotic than Caulín bay (45%, CI: 23.0–68.4%) (Fisher's Exact test, p = 0.04). Further, Caulín sediments showed only intermediate phenotypes, all against CXM. In contrast, those from Pullao also showed ARB against OXT (2/38), ENR (4/38), CFP (1/38), CXM (25/38), and CN (1/38). Multi-resistant phenotypes were observed only at Pullao bay. ARB in sediments were identified mostly as *E. coli*, followed by *Bacillus* sp. and *Staphylococcus* sp. (Table 1). Bacteria colonies with ARGs to, at least, one antibiotic were similarly present in sediments at both bays (Chi-square = 0.57, df = 1, p = 0.44), with no significant differences on the number of multi- and mono-ARGs (Chi-square = 0.73, p = 0.39). Matching ARB

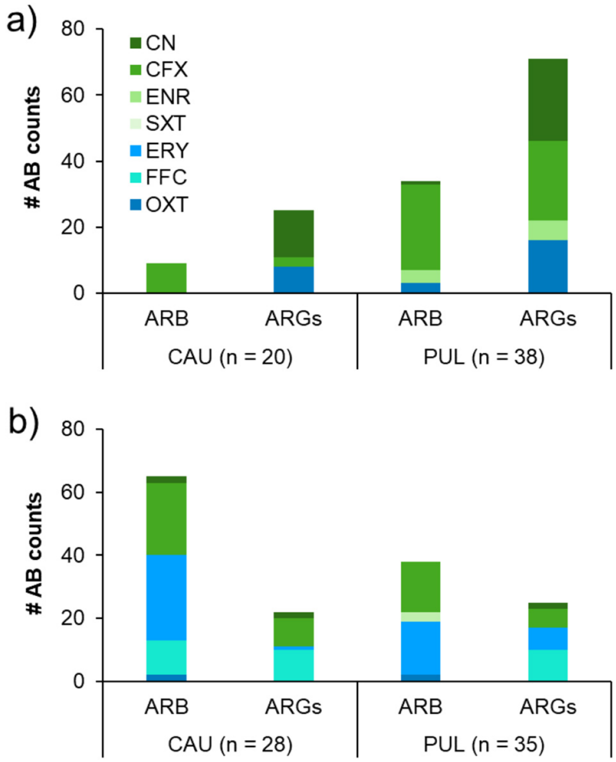

Fig. 2. Counts of phenotypical Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARGs) to eight (CFX = CFP & CXM see below) antibiotics (ABs: Blue = used in Salmon Aquaculture; green = Medical/Vet use) in (a) sediment samples and (b) cloacal samples of Hudsonian godwits *Limosa haemastica* captured at two bays (CAU and PUL) within Chiloé Archipelago (42°S, Chile). Oxytetracyclin (OXT), florfenicol (FFC) and erythromycin (ERY) from aquaculture; Sulphamethoxazole/trimethoprim (SXT), enrofloxacin (ENR), cefoperazone (CFP), cefuroxime (CXM) and gentamicin (CN) from Medical/Veterinarian sources. Sample size is indicated between brackets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

findings, Pullao bay (63%, CI: 45–78%) showed significantly higher prevalence of bla_{TEM} genes than Caulín bay (15%, CI: 3.2%–37%, Pearson's Chi-square = 12.21, df = 1, p = 0.0004) (Fig. 2a). Besides these specific differences, counts of ARB (Chi-square = 0.04, df = 1, p = 0.85) and ARGs (Chi-square = 0.45, df = 1, p = 0.50) from aquaculture and medical/veterinarian sources in sediments were similar among bays.

3.2. Birds

An 87.3% of the cloacal bird samples (55/63) showed ARB against, at least, one antibiotic (Fig. 2b). Of those, 63% (35/55) were colonies with multi-resistant ARB. A 80% (44/55) and 67% (37/55) were against ERY and CXM, respectively. Contrary to sediment findings, Caulín bay (100%, 87.6–100%) presented significantly more birds holding ARB than birds from Pullao bay (77%, 59.8–89.5%) (Fisher Exact test, p = 0.006). Further, 86% (24/28) bird samples from Caulín bay were considered multi-resistant, compared to 31% (11/35) in Pullao bay. The most common combination of multi-resistant phenotypes in birds was against ERY, CXM and FFC (11/24) in Caulín bay, while ERY and CXM was in Pullao bay (8/11). The taxa with a higher occurrence were *Enterococcus* and *Psychrobacter* sp., all of them mono- or multi- resistant (Table 1). In addition, we isolated two samples containing bacteria of genera with a high potential pathogenicity, non-resistant *Salmonella* sp. and mono-resistant *Staphylococcus* sp. (Table 1). ARGs evaluated were present in 46% (29/63) of the samples from birds at both bays, with no significant differences between bays on the frequencies of ARGs (Chi-square = 0.003, df = 1, p = 0.95). A 51% of the samples with ARGs contained genes against two or more antibiotics, with no significant differences in the frequencies of multi-resistant samples among bays (Chi-square = 0.04, p = 0.83) (Fig. 2b). A 23% of the samples showed the FloR gene and other 22% were positive to bla_{TEM} gene at both bays, being the most common multigene association. No resistant genes against OXY and SXT were found. Interestingly no significant differences were found among bays in the frequency of any of the genes evaluated. Finally, counts of ARB (Chi-square = 0.88, df = 1, p = 0.35) and ARGs (Chi-square = 0.92, df = 1, p = 0.34) from aquaculture and medical/veterinarian sources in birds were similar among bays.

4. Discussion

ARB and ARGs are consistently present both in sediments and microbiota of migratory shorebirds in Chiloé. ARB and ARGs occurrence was similar either from aquaculture and medical/veterinarian sources at

Table 1
DNA-Identification and occurrence of isolated bacteria from sediments (n = 58) and cloacal swabs of Hudsonian godwits (n = 63) captured in Chiloé Archipelago, after incubating in BHI media and exposed in XLD blood agar plates to a panel of eight antibiotics (ABs) of different family compounds used in aquaculture and medical/veterinarian treatments. The proportion of each bacteria showing phenotypic resistance to one AB (mono-resistant) and two or more ABs (multi-resistant) is shown.

Sample	Order	Taxon	Occurrence (%)	Relative AB resistance (%)	
				Mono-resistant	Multi-resistant
Sediments	Enterobacteriales	*Escherichia coli*	50	59	14
		Proteus sp.	7	25	–
	Lactobacillales	*Enterococcus* sp.	2	–	100
	Bacillales	*Staphylococcus* sp.	17	20	10
		Bacillus sp.	19	64	9
	Aeromonadales	*Aeromonas* sp.	2	100	–
	Vibrionales	*Vibrio* sp.	3	100	–
Birds	Enterobacteriales	*Escherichia coli*	13	83	–
		Pantoea sp.	7	33	33
		Citrobacter sp.	4	50	50
		Enterobacter sp.	2	100	–
		Proteus sp.	4	100	–
		Klebsiella sp.	2	–	100
		Yersenia sp.	2	100	–
		Salmonella sp.	4	–	–
	Lactobacillales	*Enterococcus* sp.	25	36	56
	Pseudomonadales	*Psychrobacter* sp.	19	67	22
	Bacillales	*Kurthia* sp.	8	75	25
		Staphylococcus sp.	4	100	–
	Actinomycetales	*Arthrobacter* sp.	2	100	–
		Curtobacterium sp.	2	–	–
	Alteromonadales	*Shewanella* sp.	2	100	–

two bays with contrasting main human activities. However, small sample sizes likely prevent reaching statistical significance among bays, since specific results indicate a more intensive antibiotic resistance selection in the bay directly influenced by aquaculture operations. In addition, our results also reinforce previous findings of ARGs (against antibiotics used in salmon aquaculture) in sediments far away from aquaculture areas (Cabello, 2006; Buschmann et al., 2012), highlighting that the antibiotic footprint can extend beyond the geographical influence of antibiotic sources and transfer to wildlife.

Recent studies showed a high occurrence of different antibiotic residues and ARGs in aquatic environments in China (Huang et al., 2019; Zhu et al., 2017), in areas with very high human population densities. However, we found that presence of ARB and ARGs at the coastal environment is ubiquitous even at one of the lowest densely populated geographic area (Patagonian coasts) and, more important, widely present at gut microbiota of migratory shorebirds (ARB: 87% samples; ARGs: 42% samples). Multi-resistant phenotypes in sediments were only found in the bay surrounded by aquaculture operations, as well as a higher ARB and ARGs occurrence. By contrast comparatively higher multi-resistant phenotypic ARB was found in birds captured at a bay located 20 km away from any salmon aquaculture development -but most probably reached by antibiotic residuals drained by relevant current speed associated to tidal cycles in Chacao channel ($4\ \mathrm{m \cdot s^{-1}}$; Narváez et al., 2019) after more than twenty year of antibiotic use in Reloncaví sound (Buschmann et al., 2012), with the most common association including resistance to two out of three antibiotics most used in aquaculture in Chile. These findings suggest that ARB harbor ARGs that can persist for long periods in aquatic environments (Taylor et al., 2011) boosting exchange of ARGs not only between marine environmental bacteria (e.g. Alteromonadales, Vibrionales; Buschmann et al., 2012) but also among Enterobacteriaceae, such that highly mobile predators as godwits using nearby bays can act as reservoirs and spreaders of antibiotic resistance.

Besides the limitations of our study, it is thus plausible that widespread ARB present in sediments were transferred to shorebirds that forage intensively at Chiloé intertidal areas during 5–6 months a year (Navedo et al., 2019), explaining why nearly all (90%) cloacal samples were mono- or multi-resistant. In addition to the physiological effects of potential antibiotic residuals consumption by predators (Noverr et al., 2005), further studies could show the extent to which those ARB and ARGs are driving changes in gut microbiota composition involved in different crucial functions related to bird fitness (Grond et al., 2018). Further, the majority of isolated taxa from birds belong to common gut microbiota in vertebrates, including humans. We have identified the occurrence of ARB with high clinical relevance both in sediments and birds, and high occurrence of ARB showing multi-resistance that are common in human microbiota. These findings highlight the potential transference of pathogens between wildlife and humans (Hernández et al., 2013) and reinforce the role as reservoirs and potential spreaders of antibiotic resistance to these birds.

We did not measure occurrence of antibiotic residuals in intertidal sediments and bird tissues. Notwithstanding this potential caveat, both sediments and birds share similar ARB and ARGs, particularly *bla*$_{\mathrm{TEM}}$ and FloR genes in godwits' microbiota, thus providing some evidence of the antibiotic footprint in the local environment where godwits forage exclusively at intertidal areas for several months each year (Navedo et al., 2019). Furthermore FFC is the most used antibiotic in salmon aquaculture, and Chiloé is the unique coastal area regularly inhabited by these migratory species once they depart from Alaskan after the breeding season (Senner et al., 2014). In fact, godwits staging in the Canadian prairies and high-amazon floodplains (see Fig. 1a) before reaching Chiloé would be exposed to pesticides used in agriculture (e.g. Malaj et al., 2020) rather than antibiotics, especially those used in coastal areas. By contrast, hundreds of ABs that have been released annually during the last decades in the coasts of Chile (SERNAPESCA, 2017), particularly in Chiloé area (Barton and Floysand, 2010), allowing the development of antibiotic resistance in sediments and raising concerns about human well-being (Cabello et al., 2016). Therefore, salmon aquaculture seems to promote the current high occurrence of FloR genes among other ARGs (see also Quiñones et al., 2019), not only in intertidal sediments but in associated wildlife microbiota by means of migratory shorebirds. In addition, our results also highlight the widespread occurrence of *bla*$_{\mathrm{TEM}}$ genes (medical/veterinarian source) in ARB from sediments and wildlife microbiota in Chiloé, with a very high occurrence (>85% samples) in a bay with a very low human density surrounding an aquaculture area.

5. Conclusions

We demonstrate the extent of multi-source antibiotic footprint in a sparsely-populated coastal area at a global scale with high conservation value for essential components of ecosystem functioning (Bauer and Hoye, 2004). Further research will disentangle whether or not these 'sentinels' of intertidal areas (Mathot et al., 2018) are experiencing changes in their fitness driven by ABR and ARGs burden in their microbiota. As potential spreaders of disease, surveillance of ARB and ARGs in main staging areas of godwits (e.g. mid-continental US) flying from Chiloé to breeding grounds in Alaska, should be carried out. In the meantime, our results lead to a consideration of antibiotic footprint by applying the precautionary principle when managing endangered species (Prato, 2005), such as godwits and closely related *Numenius* spp. that are worldwide threatened (Pearce-Higgins et al., 2017). Finally, our results are clearly conservative. On the one hand, sediments closer to salmon aquaculture operations would contain a higher amount of antibiotic residuals, and thus boosting the antibiotic footprint. On the other, using specific media for marine bacteria growth will probably identify several other ARB present at sediments holding ARGs (Buschmann et al., 2012). In spite of these limitations, our results obtained from microbiota of wildlife inhabiting bays surrounded by less than 20 people per square kilometer could show the 'tip of the iceberg': a silent pollution in many coastal areas in the austral hemisphere with higher human-population, weak environmental laws and, even more important, virtual absence of enforcement of compliance (Navedo, 2018).

Hence urgent measures are: (i) surveying the extent to which antibiotic residuals are present at important coastal areas for biodiversity conservation from different sources; (ii) monitoring and isolating ARB, and identifying ARGs harbored by migratory shorebirds throughout time; and (iii) assessing detrimental effects of potential changes on microbiota by acquired resistant bacteria. Considering antibiotic footprint into WHO and conservation policies will be especially relevant in two large geographic areas: southern South America because of the current expansion of salmon aquaculture from Chiloé within austral Fjords up to Cape Horn (Niklitschek et al., 2013) and Yellow Sea area since it receives large antibiotic residuals (Du et al., 2017) and serves as a major migratory hub for shorebirds at that Flyway (Piersma et al., 2016). Identifying main antibiotic sources at each important area to further limit their inputs, would be an initiative to serve both for the conservation of mobile species (Runge et al., 2014) and a measure for human well-being under a 'One Health' approach (Cunningham et al., 2017).

Ethics statement

All bird captures and handling procedures were carried out under the approval of the Bioethics Subcommittee of Universidad Austral de Chile (no 260/2016). All methods were carried out in accordance with these approved guidelines and regulations.

This study was supported by FONDECYT grant to J.G.N. (No. 1161224).

CRediT authorship contribution statement

J.G.N. & C.V. designed the research and collected the samples. V.A. & C.V. assessed phenotypic and genotypic resistance and performed lab analyses. J.G.N. & C.V. analysed the data. J.G.N. wrote the manuscript with contributions and revisions by all authors. All authors approved the final manuscript.

Declaration of competing interest

Authors declared no competing interest.

Acknowledgements

We thank Jorge Ruiz, all students and colleagues at www.birdecologylab.cl for fieldwork support, and Ecology & Evolution of Infectious Diseases Lab for laboratory support. Mark Drever helped with English edition and Isabel Guerrero designed Fig. 1. Comments made by four anonymous reviewers helped to improve the manuscript. Captures and sampling were made under bioethics approval #260/2016 from Universidad Austral de Chile and permission #5932/2016 from SAG/Gobierno de Chile. This work was funded by FONDECYT grant #1161224 (JGN).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146004.

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