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Human-associated Extended Spectrum β-Lactamase (ESBL) in the Antarctic

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Abstract

Escherichia coli with extended spectrum β-lactamase (ESBL) type CTX-M resistance were isolated from water samples collected close to research stations in Antarctica. The isolates had $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ genotypes and sequence types (ST) indicative of a human-associated origin. This is the first record of ESBL-producing enterobacteria from Antarctica.

Enterobacteriaceae with extended spectrum β-lactamase (ESBL) enzymes is increasing in clinical and veterinary medicine, posing a threat to future healthcare (6). ESBL-producing bacteria are now commonly isolated both in industrialized and in developing countries (6, 12), and dissemination into the environment has been observed (1). The most common ESBLs are TEM, SHV and CTX-M – each with a different evolutionary origin (4, 9). These classes are subdivided into gene-groups and to date >300 different ESBL variants have been described (8). In recent years the CTX-M has become the most widespread class (5). The Antarctic continent is the last comparative pristine ecosystem with a small human population restricted to research bases, primarily located on the Antarctic Peninsula. Human activities are regulated by the Antarctic Treaty to reduce interference with the unique wildlife and the impact of human-associated microorganisms should be minimal (2). However, contrary to this intention, human-associated pathogens have been indentified in Antarctic wildlife (18). Here we report the presence of human associated ESBL-producing *Escherichia coli* with bla_{CTX-M} genes in water sampled close to research bases on the Antarctic Peninsula and the South Shetlands Islands, providing the first cases of ESBL in the region.

The fieldwork was conducted during the 2011 austral summer (January-February)when the bases Bernardo O´Higgins on the Antarctic Peninsula, the Fildes Bay (King George Island) and the Arturo Prat (Greenwich Island) in the South Shetlands Islands were visited for sampling. Water samples were collected from the sea surface in concentric circles from the stations' sewage outlets (10, 25, 50, 100, 200 and 300 m). Each of the 123 water samples (125 ml volume) was filtered through sterile 0.45 µm membrane filters which were cultured on chromogenic selective plates (ChromoCult, Merck, Darmstadt, Germany) for detection of *E. coli* and coliform flora. The filters were then placed in Luria broth (phosphate-buffered saline including 0.45% Na-citrate, 0.1% $MgSO_4$, 1% $(NH_4)_2SO_4$ and 4.4% glycerol) and stored at -70°C. Additionally, we sampled a total of 400 fresh faecal

droppings from gentoo penguins (*Pygoscelis papua)* from colonies close to the bases. All samples were shipped on dry-ice to Sweden for further analyses.

In order to isolate *E. coli* for characterization, all water and penguin samples were cultivated on Uriselect4 plates (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK) and colonies were identified by conventional biochemical tests. Randomly chosen 71 *E. coli* isolates (40 from water and 31 from penguin samples) to determine antibiotic susceptibility profiles using the EUCAST disc diffusion method in order to receive an overall picture of resistance in the material (15). The panel included 11 antibiotic discs: tetracycline 30 µg/disc, ampicillin 10 µg/disc, streptomycin 10 µg/disc, chloramphenicol 30 µg/disc, nalidixic acid 30 µg/disc, cefadroxil 30 µg/disc, fosfomycin 50 µg/disc, tigecycline 15 µg/disc, trimethoprim/sulphamethoxazole 1.25/23.75 µg/disc, nitrofurantoin 100 µg/disc and mecillinam 10 µg/disc (all antibiotics from Oxoid Ltd, Cambridge, UK). The *E. coli* strain ATCC25922 was used as control.

Only one *E. coli* isolate from a penguin (Bernardo O´Higgins base) had a resistant phenotype, in this case to chloramphenicol. In the water samples, three isolates were resistant to at least one antibiotic compound and several were resistant to two or more antibiotics (Table 1). The most frequently observed resistance was to ampicillin, found in eleven isolates, followed by tetracycline (six isolates), streptomycin (four isolates), and trimethoprim/sulphamethosaxole (four isolates). One *E. coli* was resistant to nalidixic acid (Table 1). The presence of ESBL-producing bacteria was investigated by enriching all samples in Brain Heart Infusion broth (Becton Dickinson, Franklin Lakes, USA) supplemented with vancomycin (16 mg/L, ICN Biomedicals Inc. Aurora, USA) for 18h at 37°C, and subsequently inoculate on chromID™ ESBL plates (bioMérieux, Marcy L´Etoile, France). Ten *E. coli* ESBL isolates were retrieved and ESBL production was confirmed in each isolate with a cefpodoxime/cefpodoxime + clavulanic acid double disk test (MAST

Diagnostics, Bootle, UK). These isolates were all positive for the CTX-M ESBL but negative for TEM and SHV in specific qPCR (13, 14). The PCR products were sequenced on both strands using the following primers: CTX-F (5`TCCCAGAATAAGGAATCCCAT-3`) and CTX-R1 (5`CCCATTCCGTTTCCGCTA-3`). The resulting consensus sequences were compared with published sequences in the NCBI database. Four *E. coli* isolates, all from Fildes Bay, carried the $bla_{\text{CTX-M-1}}$ gene, while sex isolates (four from Arturo Prat and two from Bernardo O´Higgins) carried the $bla_{\text{CTX-M-15}}$ gene. The ESBL isolates were further analysed by multi locus sequencing typing (MLST) using a modified version of previously described procedures (19). Sequence types (STs) and putative associated sources were retrieved from an on-line MLST *E. coli* database (http://mlst.ucc.ie/mlst/dbs/Ecoli/). Six different STs (ST131, ST227, ST401, ST410, ST685 and ST937) two to four at each research station were identified (Table 2).

The $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ genes are both human-associated ESBL genotypes (16, 19). Genotyping by MLST corroborated the association with human gastrointestinal biota, as the majority of the STs belonged to widespread disease-associated genotypes, including *E. coli* ST131 carrying $bla_{\text{CTX-M-15}}$ gene, a worldwide disseminated human clinical clone (11).These findings constitute the first records of ESBL-producing bacteria in Antarctica, and given that they were isolated from all the sampled bases it seems that human-associated bacteria are discharged into the environment more than occasionally. The emergence of CTX-M has been referred to as one of the most striking examples of rapid, global dissemination of plasmid-mediated resistance determinants among bacterial pathogens (16). Nosocomial and community spread of ESBL genotypes are well described in humans (3) but there are less studies on the presence of these genes in bacteria in the environment (1). The efforts to suppress further development and dispersal of resistance are focused on reducing the consumption of antibiotics. However, the factors that maintain, and perhaps

facilitate further resistance dispersal in natural environments (e. g. sewage treatment) are given less attention. The mechanisms behind the success of CTX-M are still to be determined (16). Therefore, the finding of CTX-M-producing bacteria in Antarctic seawater and the finding of high prevalence of CTX-M in certain wild bird populations needs to be considered carefully. In addition to ESBL-producing isolates, several non-ESBL *E. coli* with resistance to common antibiotics were found in the water samples, including isolates with resistance to up to 4 compounds (Table 1). An important question to ask is whether the human-associated bacteria found close to human activities infect Antarctic wildlife. None of the sampled penguins were positive for ESBL-producing bacteria, and only one *E. coli* exhibited antibiotic resistance, indicating that at the time of sampling human-associated enterobacteriaceae was not common in the sampled penguins. The risk of a bird to be colonized by *E. coli* has been shown to vary with the proximity to environments influenced by human activity (10). An earlier study in Antarctica indicated a 17 % prevalence of *E. coli* in penguins (17). The lower prevalence of *E. coli* (~8%) in our samples could indicate infrequent interactions between penguin and human microbiota, especially since the penguin strains were highly susceptible to antibiotics and showed no presence of ESBL. However, penguins are but one part of the ecosystem, and compared to more opportunistic feeders, such as kelp gulls (*Larus dominicanus*), skuas (*Stercorarius* spp.) and snowy sheatbills (*Chionis albus*), they may be less exposed to human-associated bacteria. An opportunistic diet has previously been associated with the presence of human-associated enteropathogens in birds (7). At present, we cannot tell whether ESBL-producing bacteria are present in Antarctic wildlife, or the consequences that would have for animal health. However, the presence of anthropogenic bacteria in the Antarctic environment is worrisome in itself and indicative of how widespread the global antibiotic resistance situation has become. The existing precautions and sewage treatment at the research bases seem inadequate. Clearly, increased sampling in the Antarctic

biome is warranted, as well as increased efforts in reducing potential leakage of bacteria from human activities.

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Table 1: The number of resistant *E. coli* isolates from seawater samples collected at three research bases in the Antarctic region, 2011.

Antibiotic compound	Number of *E. coli* isolates per location		
	Greenwich Island	Antarctic Peninsula	King George Island
Nalidixic Acid	0	0	1
Streptomycin	1	1	2
Tetracycline	5	0	1
Ampicillin	7	2	2
Trimethoprim/Sulphametoxasole	1	1	2
Susceptible to all compounds	0	8	20
Resistant to 1 compound	1	1	1
Resistant to 2 compounds	5	0	0
Resistant to 3 compounds	1	1	1
Resistant to 4 compounds	0	0	1
Total	**7**	**10**	**23**

Table 2: Genotypic and phenotypic characteristics of ESBL-producing *E. coli* isolates from water samples collected at three research bases in the Antarctic region, 2011.

Location	ESBL genotype			MLST		Phenotypic Resistance
	CTX-M	TEM	SHV	ST	Clonal Complex	
Greenwich Island	-15	-	-	ST410	ST23 complex	Na[1], Te[2], Amp[3], Cfr[4]
	-15	-	-	ST410	ST23 complex	Na, Te, Amp, Cfr
	-15	-	-	ST410	ST23 complex	Na, Te, Amp, Cfr
	-15	-	-	ST685	Unassigned	Na, Amp, Cfr
Antarctic Peninsula	-15	-	-	ST937	Unassigned	Na, Te, Amp, Cfr
	-15	-	-	ST131	Unassigned	Na, Te, Amp, Cfr
King Georges Island	-1	-	-	ST401	Unassigned	Amp, Cfr
	-1	-	-	ST131	Unassigned	Amp, Cfr
	-1	-	-	ST410	ST23 complex	Amp, Cfr
	-1	-	-	ST227	ST10 complex	Amp, Cfr

[1]Nalidixic Acid (Na), [2]Tetracyclin (Te), [3]Ampicilin (Amp), [4]Cefadroxil (Cfr).