REVIEW ARTICLE

Anthropogenic antibiotic resistance genes mobilization to the polar regions

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Anthropogenic influences in the southern polar region have been rare, but lately microorganisms associated with humans have reached Antarctica, possibly from military bases, fishing boats, scientific expeditions, and/or ship-borne tourism. Studies of seawater in areas of human intervention and proximal to fresh penguin feces revealed the presence of *Escherichia coli* strains least resistant to antibiotics in penguins, whereas *E. coli* from seawater elsewhere showed resistance to one or more of the following antibiotics: ampicillin, tetracycline, streptomycin, and trim-sulfa. In seawater samples, bacteria were found carrying extended-spectrum β-lactamase (ESBL)-type CTX-M genes in which multilocus sequencing typing (MLST) showed different sequence types (STs), previously reported in humans. In the Arctic, on the contrary, people have been present for a long time, and the presence of antibiotic resistance genes (ARGs) appears to be much more wide-spread than was previously reported. Studies of *E coli* from Arctic birds (Bering Strait) revealed reduced susceptibility to antibiotics, but one globally spreading clone of *E. coli* genotype O25b-ST131, carrying genes of ESBL-type CTX-M, was identified. In the few years between sample collections in the same area, differences in resistance pattern were observed, with *E. coli* from birds showing resistance to a maximum of five different antibiotics. Presence of resistance-type ESBLs (TEM, SHV, and CTX-M) in *E. coli* and *Klebsiella pneumoniae* was also confirmed by specified PCR methods. MLST revealed that those bacteria carried STs that connect them to previously described strains in humans. In conclusion, bacteria previously related to humans could be found in relatively pristine environments, and presently human-associated, antibiotic-resistant bacteria have reached a high global level of distribution that they are now found even in the polar regions.

Keywords: *polar regions*; *human-associated bacteria*; *antibiotic resistance genes*; *wildlife*; E. coli; *K. pneumoniae*; *antibiotics*; *ESBL*; *CTX-M*; *Enterobacteriaceae*

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Consequences that introduced microorganisms have in the Antarctic and Arctic environment and their wildlife are so far unexplored. Until 1820, the Antarctic continent had been untouched by humankind and was considered one of the last unexplored areas in the world. Today, we know that human-associated microorganisms have reached the Antarctic continent because of military bases, fishing boats, scientific programs, and increased tourism (1). In the Arctic, there is a close connection between human communities and wild terrestrial and marine mammals as an important part of sustenance and cultural expressions.

Migratory birds can be implicated in dissemination of microorganisms as biological or mechanical carriers (2) and in that way act as transporters between different geographical areas (3). Some birds migrate to the Antarctic during the austral winter and could pick up bacteria a long their regular pathways (4). For this reason, birds that reside on or overwinter in Antarctica can be used as biomarkers to determine the presence of microorganisms in the environment (5). Previous studies have shown the presence of antibiotic-resistant bacteria in birds migrating through Europe and also in their migratory routes extending to the Arctic (6). Studies of Antarctic birds have also revealed the presence of human-associated bacteria such as *Campylobacter* spp. and *Salmonella* spp. (4, 5), and they have also been isolated from penguins and seals from sub-Antarctic islands (7, 8). *Escherichia coli* and *K. pneumoniae* carrying antibiotic resistance genes (ARGs) can transfer these to other bacteria by conjugation

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mechanisms both within and between bacterial species (9). The bacteria that become resistant also inherit the ability to spread these genes to other bacteria (10, 11). In a period of few decades, the number of bacterial species resistant to antibiotics has notably increased (12). In urban areas, humans generate conditions for the founding and spread of infectious diseases among animals and people by agricultural activities, including livestock by increasing human contact with animal waste and butcher waste (13, 14). For a long time, antimicrobials have been used as a growth factor in animal production and also as prophylactics in livestock raising (15). Treatment of food-producing animals with antibiotics may become a public health risk by the transfer of selected resistant zoonotic bacteria from animals to humans (16). A well-described phenomenon associated with the use of antibiotics is the increasing prevalence of resistance of some of the most common bacteria in humans (9, 17, 18). One example is the β-lactam antibiotics and the emergence of multiresistance that are considered to be a direct consequence of animals feed supplementing with antibiotics (19).

Wild birds can be colonized by different bacterial strains, including human-associated pathogens. ARGs have been found in bacteria from wild bird species, including mallards (*Anas platyrhynchos*), herring gulls (*Larus argentatus*) and other aquatic birds in the Baltic Sea region (20–22), Germany and Mongolia, and in black kites (*Milvus migrans*), red kites (*Milvus milvus*), black vultures (*Aegyptus monachus*), and demoiselle cranes (*Anthropoides virgo*) (23). Czech, Swedish, German, and Portuguese studies have shown that bacteria isolated from wild birds predominantly harbor extended-spectrum β-lactamase (ESBL) genotypes. Human intestinal bacteria can be found in a variety of environments such as soil, vegetation, and water and have been identified as a major component of the microflora in several non-clinical environments (24), and have become involved in nosocomial infections (25). In animals, those bacteria are mostly associated with sepsis, urinary and respiratory tract infections, and mastitis (26, 27). Several of the most important members of the family Enterobacteriaceae are becoming progressively resistant to antibiotics (11). Antibiotic resistance is a current but not a new phenomenon. Recent studies have reported the presence of genes that encode resistance to modern variants of antibiotics from a 30,000-year-old Arctic permafrost sediment (28) and in bacteria from a cave in New Mexico that has been isolated for over 4 million years (29).

The enzyme that hydrolyzes expanded-spectrum cephalosporin, ESBL-type CTX-M, was acquired by bacteria that can cause infections in humans and took its genes from environmental *Kluyvera* spp. (30). As a consequence of antibiotic selection pressure, ARGs genes have been mobilized between environmental microorganisms, humans, and other animals' bacterial flora, including birds, and probably in the reverse direction (31–33). Geographically widespread epidemic clones with the same chromosomal sequence types (STs) have been identified among *E. coli* strains. The efficacy of β-lactam antibiotics is continuously tested by the emergence of new and broadly resistant bacterial strains (34). ESBLs are enzymes that inactivate broad spectrum β-lactam antibiotics of recent generation. The term *extended-spectrum β-lactamase* was coined by Philippon in 1989 (35), and ESBLs are defined as β-lactamases with following characteristics: they are transferable; they can hydrolyze penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins); and they can be inhibited *in vitro* by β-lactamase inhibitors such as clavulanic acid. Most ESBL variants are derived from the traditional TEM or SHV β-lactamase enzymes, often found in *E. coli* and *K. pneumoniae*. In the recent decades, the CTX-M enzymes have become more prevalent and frequent than the TEM and SHV enzymes (36–38). The CTX-M enzymes are not closely related to the traditional TEM or SHV enzyme since they have a high homology with a chromosomal enzyme from an environmental bacterium of the genus *Kluyvera*. The explosive dissemination of CTX-M around the world has now reached a pandemic level (36, 39). Enterobacteriaceae producing ESBL, in particular the CTX-M type, is a major problem worldwide, causing outbreaks as well as sporadic infections (40). The emergence and wide dissemination of the CTX-M-15 enzyme is one of the most relevant findings associated with the current epidemiology of ESBL. Recent studies have demonstrated that the highly virulent *E. coli* O25:H4-ST131 is responsible for the pandemic dissemination of the CTX-M-15 enzyme (41). Plasmids carrying the $bla_{CTX-M-15}$ gene are not exclusive to clone ST131 since they have been identified in other *E. coli* STs such as ST405, ST354, ST28, and ST695. The significance of human-associated bacteria or ARGs mobilization in the Arctic and Antarctic wildlife and environment is to date an open question.

Mobilization of ARGs to Polar Regions

The Arctic and Antarctic regions are rich and diverse ecosystems for birds, fish, and mammals. The short summer and long winter forces many animals to be migratory. This in turn means that there is a risk that migratory birds (and other animals) can bring pathogens or resistant bacteria into animal populations in the Arctic and Antarctic environments (42). Whether the introduction of antibiotic-resistant bacteria is a hazard to the ecosystems remains to be elucidated. Yet the number of tourists visiting the polar regions is continuously increasing, which raises the risk of introducing microorganisms (7, 43). The consequences that human-associated microorganisms, pathogenic or not, may have on the environment and the local wildlife in the polar regions are difficult

to predict. The only continent without ESBL-producing bacteria until now was Antarctica (44).

In the Arctic, industrial development has been intense and has resulted in substantial impacts while the region has also been strategic militarized territory. The Arctic has a population of about 3.8 million inhabitants, and the environment has been under external stress for considerable amount of time (45). The local biota has been exposed to anthropogenic pressure and is considered particularly vulnerable to alterations (46). In one of the few studies from the Arctic, it was shown that bacteria from migratory shorebirds from the Siberian and Alaskan tundra displayed antibiotic resistance, suggesting that these bacteria have reached broad geographic dissemination (6). Probably the birds picked up the bacteria in their wintering grounds and brought them to their breeding grounds.

In Antarctica, however, international agreements regulate the number of visitors, researchers, or tourists who can be present at the same time (47). One additional factor is the waste from Antarctic research bases, military bases, fishing boats, scientific programs, and tourism. The question about leakage from the stations' sewage is not sufficiently known. When inadequately treated liquid waste spills into the seawater, human-associated bacteria may become introduced into the environment (43). Before 1990, all the waste from Antarctic research stations were dumped in landfill sites located close to the bases, or were discarded into the sea (48). The low water temperature allows certain bacteria to survive in the environment for a relatively long time (8). Fecal bacteria can survive a few minutes to several days in the seawater, depending on temperature, solar radiation, and salinity; in Antarctica these factors change dramatically according to the season (49). Another possibility is the introduction by birds, as there are species such as kelp gulls (*Larus dominicanus*) and other sporadic visitors that during non-breeding time may reach densely populated areas in southern South America during the polar winter (2, 3, 50, 51). Human-derived bacteria have been documented in sewage outlets and waste from the Antarctic stations, and the local fauna has accidentally been infected by contamination from sewage.

Discussion

The finding of vancomycin-resistant *Enterococcus* (VRE) in glaucous gulls from Alaska (52) in 2005 showed that antibiotic-resistant bacteria and antibiotic resistance genes (*vanA*) had already reached remote areas. Outbreaks by VRE strains can possibly arise in hospitals when already well-established vancomycin-sensitive *E. faecium* strain acquire vancomycin resistance genes via a resistant plasmid (53). The finding of similar VRE bacteria belonging to the same clonal cluster (CC17) was described internationally as well established in hospital environments. The glaucous gull is a bird that has a circumpolar distribution

and is short distance migrator from the North Atlantic to North Pacific Ocean. This species is a regular visitor of urban environments such as city dumps and sewage outlets close to human habitats (54, 55). Five years later, in 2010, VRE was found in the same bird species in the same place (56). The current isolates harbored both *vanA* and *esp* genes and belonged to the same lineage found in 2005 (52, 56). The long-distance spreading of VRE is probably not as a result of migratory birds. It is more likely that the birds are indicators of VRE in the environment and that the birds act as a short distance or local disseminators. The situation contrasts with *Enterococcus* and Enterobacteriaceae resistance in the sub-Arctic area. Despite the low resistance to antibiotics demonstrated in Arctic bird bacteria in 2005, ESBL-producing *E. coli* in the Kamchatka peninsula and in the Commodore islands were detected. *E. coli* of two different ESBL-producing strains were detected, and these carried ESBL-type CTX-M genes $bla_{ctx-m-14}$ and $bla_{ctx-m-15}$. One isolate belonged to the globally disseminated ESBL *E. coli* O25b-ST131 clone. This strain was found in glaucous-winged gulls at Commander Island, which may be a consequence of the successful transfer of the pandemic ST131 *E. coli* clone of human origin to the environment (57). ESBL-carrying bacteria isolated from birds illustrate the presence of resistance genes in the sub-Arctic area. In summer 2010, feces samples from glaucous gulls were collected in Barrow, AK, USA. The results showed the occurrence of antibiotic-resistant bacteria of Enterobacteriaceae with the ESBL types CTX-M, TEM, and SHV, either in single or combined form. Bacteria that harbored ESBL were found in 34.1% of samples, and were both *E. coli* and *K. pneumoniae*. In *E. coli*, both CTX-M and TEM were detected, and *K. pneumoniae* had all CTX-M, TEM, and SHV variants. The multilocus sequencing typing showed different STs in *E. coli* that are correlated to previously described clinical isolates in humans. ESBL-producing bacteria, principally the CTX-M variant, are no longer just a problem in medical facilities. Currently, they are spreading in the community and the environment (58). *E. coli* ST13 (O25b:H4), associated with the CTX-M-15 ESBL, are probably one of the most important and predominant in human infections and were also found in *E. coli* in the analyzed Arctic material, which means that this antibiotic-resistant type has spread globally. Antibiotic susceptibility testing in ESBL-carrying bacteria revealed differences between the two species: all *E. coli* were resistant to six of the 11 compounds while all *K. pneumoniae* were resistant to three to eight compounds (56). ESBL-carrying bacteria were not present in the material sampled by the Swedish Arctic expedition, 'Beringia 2005', but were found in a large numbers in Barrow in 2010. Remarkable is the presence of ST10, ST38, ST131, and ST405 in *E. coli* which are the STs responsible for the dissemination of CTX-M worldwide. This is an indication that the ESBL

did not emerge spontaneously in the Alaska environment. The fact that these ESBL genotypes are found in remote parts of Alaska strongly supports the theory that they spread from urbanized areas to more pristine environments. Birds in general are good bioindicators that reflect the presence of microorganisms in the ecosystems, in particular under non-breeding periods close to urban areas with high antibiotic pressure, and the dissemination of antibiotic-resistant bacteria and resistance genes to the environment. This study has verified that antibiotic resistance genes can also be found in bacteria isolated from wild birds not exposed to significant antibiotic pressure. The bacteria that carry antibiotic resistance genes could be introduced by migratory birds or human intervention by inhabitants, tourism, and research programs in the region. In summary, in only few years between 2005 and 2010, the frequency of ESBL-harboring bacteria that can be associated with human clinical strains, in the same sampling area and in the same bird species, has increased in the region.

Antarctica is geographic isolate, with extreme ecological conditions and limited accessibility, often considered the last pristine continent. Several studies have demonstrated the presence of coliforms and other fecal bacteria since many research stations discharge human waste directly into the sea (59). Penguins can be used as biological markers to detect the presence of human-associated microorganisms or human indicator intestinal bacteria as Enterobacteriaceae (57). Up till now, the lower prevalence of *E. coli* in gentoo penguins, the highly antibiotic sensibility, and the absence of ESBL indicate a sporadic interaction between penguins and the human intestinal bacteria. On the other side, penguins are part of the ecosystem in which ESBL-carrying bacteria were found and they may be less exposed to waste water discharged from human settlements into the sea. No ESBL or antibiotic resistance was detected in penguins' samples; the presence of human intestinal bacteria carrying ESBL genes in sea water can expose penguins to contamination. Bacteria, such as *E. coli and K. pneumoniae*, found in normal bowel flora, play a crucial role in the spread of resistance genes and can act as important reservoir for ESBL-type genes. In a study published in 2016, it is concluded that naturally occurring antibiotic resistance in *E. coli* strains from Antarctic bird is rare and the bacterial antibiotic resistance found in seawater is probably associated with discharged treated wastewater (60).

Furthermore, Antarctica, is considered as an unexplored viruses, bacteria, and fungies pantry, in which the microorganisms growing under extreme conditions. These microorganisms can provide answers to the new challenges facing humanity, such as the uncontrolled antibiotic resistance development which threatens public health, as these may give rise to new antibiotic prototypes. Until now, ESBL-producing bacteria have been described in all continents except Antarctica (61). This reality has changed

with the first finding of ESBL-type CTX-M in the region (44). Antibiotic-resistant bacteria have been detected in the polar regions, but the situation is different, as the trend in the Arctic indicates an increase when comparing the findings in wildlife over an interval of 5 years, while in Antarctica the detection is only linked to human activity.

Conclusions

The results of the studies performed in the polar regions to detect the presence of human-associated bacteria and ARGs support the assumption that bird migration can be accidentally or partly involved in the spread of pathogenic microorganisms to the Arctic and/or Antarctic.

However, these studies support the notion that human-associated bacteria are mainly spread through human presence and human activities in the regions. In few years between studies in the same Artic area and same bird species, the frequency of human-associated bacteria carrying ARGs, and specifically ESBL genes, has notoriously increased. This tendency is in accord with the antibiotic resistance development in the urbanized world where new and most versatile and broadly resistant bacteria have been detected.

The overall antibiotic susceptibility of bacteria isolated from the Arctic and Antarctic samples is low in contrast to that in highly urbanized and densely populated areas.

Antibiotic-resistant bacteria carrying ESBL genes were documented in the environment adjacent to the scientific bases in Antarctica. Bacteria isolated from wildlife at the same time in the same places showed the absence of antibiotic resistance and ESBL genes. This indicates that antibiotic-resistant bacteria isolated from the Antarctic environment are mainly because of human presence and human activities in the region.

E. coli and *K. pneumoniae* associated with human clinical isolates, carrying both virulent and antibiotic resistance genes, were detected both in the Arctic and in the Antarctic regions. Those bacteria carried molecular markers linked to strains previously described in humans, which could explain the mobilization of human-associated microorganisms to the polar regions.

Certainly, antibiotic-resistant bacteria have been detected in the Arctic and Antarctic regions. However, the observed situation is radically different; the trend in the Arctic region on resistant bacteria is increasing whereas in Antarctica the presence of resistant bacteria is closely related to human activity and resistant bacteria has not been detected in wildlife.

ESBL-producing bacteria are nowadays present also in the last ESBL-free continent, the Antarctica.

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