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Escherichia coli out in the cold: Dissemination of human-derived bacteria into the Antarctic microbiome☆

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ABSTRACT

Discharge of untreated sewage into Antarctic environments presents a risk of introducing non-native microorganisms, but until now, adverse consequences have not been conclusively identified. Here we show that sewage disposal introduces human derived *Escherichia coli* carrying mobile genetic elements and virulence traits with the potential to affect the diversity and evolution of native Antarctic microbial communities. We compared *E. coli* recovered from environmental and animal sources in Antarctica to a reference collection of *E. coli* from humans and non-Antarctic animals. The distribution of phylogenetic groups and frequency of 11 virulence factors amongst the Antarctic isolates were characteristic of *E. coli* strains more commonly associated with humans. The rapidly emerging *E. coli* ST131 and ST95 clones were found amongst the Antarctic isolates, and ST95 was the predominant *E. coli* recovered from Weddell seals. Class 1 integrons were found in 15% of the Antarctic *E. coli* with 4 of 5 identified gene cassette arrays containing antibiotic resistance genes matching those common in clinical contexts. Disposing untreated sewage into the Antarctic environment does disseminate non-native microorganisms, but the extent of this impact and implications for Antarctic ecosystem health are, as yet, poorly understood.

1. Introduction

Antarctica is often perceived as a pristine ecosystem, but growth in tourism and scientific operations is rapidly increasing anthropogenic impacts across the continent (Enzenbacher, 1994; Lynch et al., 2010). Increased human visitation increases the risk of introducing non-indigenous organisms into Antarctic environments (Cowan et al., 2011). To mitigate these risks, Antarctic-bound vessels and personnel are required to follow quarantine measures. However, these measures mainly target macro-organisms such as invertebrates and plants, with less attention being given to microbes (Tsujimoto and Imura, 2012). Studies that examine possible biological introductions also target larger organisms, with microbes receiving comparatively little attention (Frenot et al., 2005; Lisle et al., 2001; Chown et al., 2012). In cases where microbial introductions have been studied, the focus has been on potential pathogens and the consequent risks to Antarctic vertebrates arising from sewage pollution (Vigo et al., 2011). Although there are numerous avenues that would facilitate the introduction of non-indigenous organisms to Antarctica, the localised and continual input of microorganisms via untreated sewage represents a significant and long-term source of non-indigenous bacteria.

Sewage pollution of aquatic environments can increase nutrient load, change pH, temperature and turbidity, and lower dissolved oxygen availability, while also polluting receiving waters with contaminants such as metals and PBDEs (Stark et al., 2015; Wild et al., 2015), and diverse and potentially pathogenic microbes. Sewage pollution disseminates mobile genetic elements and other

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genes, aiding the penetration of novel genetic material into new organisms and ecosystems (Moura et al., 2014; Czekalski et al., 2014; Shanks et al., 2013; Gillings and Stokes, 2012; Gillings, 2013). Disposal of sewage into aquatic environments can have far-reaching effects, including perturbing microbial communities in the environment and in animal microbiomes.

Methods for disposal of sewage in Antarctica vary between stations. Under the Antarctic Treaty System, the primary recommendation for sewage management is for removal to the maximum extent possible (Annex III to the Protocol on the Environmental Protection, 2005). Where removal is not possible, sewage should be disposed in accordance with criteria relating to location of station, surrounding environmental conditions, number of personnel, station type and operational periods. For example sewage cannot be disposed onto sea ice, ice shelves or grounded ice sheet, or into fresh water. Sewage can be disposed directly into the sea if the receiving environment allows initial dilution and rapid dispersion. Maceration prior to discharge is required for stations where summer personnel reach more than 30. Although sewage treatment facilities exist at many stations in Antarctica, 44 stations with year round activity (37%) do not have sewage treatment options and release untreated sewage directly into the sea (Gröndahl et al., 2009; Smith and Riddle, 2009).

Disposal of untreated sewage into Antarctic ecosystems facilitates the release of human-associated bacteria into the marine environment. *Escherichia coli*, *Salmonella enterica*, *Clostridium perfringens* and various enterococci have all been recovered in the vicinity of stations that dispose of untreated sewage (Hughes and Thompson, 2004; Gröndahl et al., 2009; Delille and Gleizon, 2003; Mcfeters et al., 1998; Delille and Delille, 2000; Howington et al., 1992). For bacterial species that are present in both humans and Antarctic wildlife, the human strains likely differ from animal strains, both genetically and phenotypically. Hence profiling target organisms provides a method for measuring introgression of microbes from human sources into Antarctic ecosystems.

Previous studies of microbial sewage pollution in Antarctica have primarily focused on detection and enumeration of indicator species that are traditionally used as measures of water quality and do not provide the discriminatory power to identify host sources (Smith et al., 1994; Lisle et al., 2001; Hughes and Thompson, 2004). *E. coli* is commonly used as an indicator of faecal pollution, and specific genotypic characteristics such as phylotyping and virulence trait carriage are increasingly being used to track faecal contamination events and host source (Leclerc et al., 2001; Power et al., 2005; Gordon and Cowling, 2003; Gordon et al., 2005). Four primary *E. coli* phylotype groups (A, B1, B2 and D) that differ in their ecological characteristics, such as host affinities, have been identified (Clermont et al., 2000). Phylogenetic group B1 are more frequently isolated from vertebrates and the environment (Gordon and Cowling, 2003). Group B2 strains are more common in mammals and of particular significance as many extraintestinal pathogens of humans, companion animals and Aves belong to this group (Clermont et al., 2014; Johnson et al., 2008). Genome sequencing and increased data on *E. coli* from multilocus sequence typing has identified additional phylogroups C, E and F (Cleremont et al., 2011; Jaureguy et al., 2008; Tenaillon et al., 2010) and cryptic lineage or clade strains (Walk et al., 2009). Virulence gene carriage also provides an indicator of host source (Gordon and Cowling, 2003; Gordon et al., 2005). Although it is thought that commensal *E. coli* may reside in the microbiota of Antarctic vertebrates, recovery of *E. coli* from penguins and marine mammals is usually low. Isolation of *E. coli* from Antarctic penguins was approximately fourfold less than that for penguins in temperate zones (Soucek and Mushin, 1970). Similarly, commensal *E. coli* strains do not appear to be common in marine mammals, from either polar or temperate regions (Johnson et al., 1998; Delport et al., 2015). Strain typing of *E. coli* from Antarctic vertebrates has not been reported.

The genetic determinants of antimicrobial resistance are also beneficial for tracking sources of microbial pollution. The mobility of DNA elements such as the integron has facilitated the rapid emergence of antibiotic resistance. Of the three classes of integrons, the clinical class 1 integron is commonly associated with resistance in human clinical settings. The clinical class 1 integron comprises a highly conserved integrase gene (*IntI1*), a recombination site (*attI*) and a promotor (*Pc*) (Gillings, 2014) which together facilitate mobility within and between bacterial species, and gene capture. The Class 1 integron is derived from a Tn402 transposon that captured the *intI1* gene and throughout its rapid emergence in association with antibiotic use the mechanistic genes of the clinical class 1 integron (*IntI1*, *attI* and *Pc*) have remained highly conserved (Stokes et al., 2006; Gillings et al., 2008b). Clinical class 1 integrons are now widespread in production animals, companion animals and wildlife. A higher prevalence of bacteria carrying clinical class 1 integrons is repeatedly observed in animal populations in near proximity to humans (Skurnik et al., 2006; Power et al., 2013; Delport et al., 2015).

Although *E. coli* has previously been isolated from Antarctic sources, these studies mostly predate recent advances in our knowledge of *E. coli* ecology, or were conducted before the development of genetic markers that enable host sources to be identified or reported on presence/absence data in alignment to water quality standards (Smith et al., 1994; Hughes and Nobbs, 2004; Smith and Riddle, 2009). One report has however examined samples from Antarctica for integrons with four *E. coli* isolates from Emperor penguins testing negative for class 1, class 2 and class 3 integrons (Skurnik et al., 2006).

For the past 10 years, Davis Station, one of four Australian Antarctic stations, has released untreated macerated sewage into the marine environment (Connor, 2008). Here we genetically characterized *E. coli* isolated from seawater, sediment and wildlife faeces collected in the vicinity of Davis Station. We performed phylotyping (Bidet et al., 2007; Gordon et al., 2005; Clermont et al., 2000), and screened for virulence determinants (Gordon et al., 2005; Johnson et al., 2008) and clinical class 1 integrons in *E. coli* isolated from Davis Station Antarctica, to address the primary aim of determining if human-associated bacteria have penetrated Antarctic microbiomes. Genetic data from a reference collection of *E. coli* from different sources in Australia was used as a comparison (Gordon and Cowling, 2003; Gordon et al., 2005).

2. Materials and methods

2.1. Study location

This study was performed at Davis Station, Princess Elizabeth Land, Antarctica (68°.35′ S 77°58′ E) during the Austral summer of 2009/2010. Davis station is situated within the Vestfold Hills, an ice-free area of the Ingrid Christensen Coast between the Sørsdal Glacier (south) and the Antarctic Plateau (north). Davis Station is a permanent Australian base supporting 80–100 expeditioners in summer and 20 in winter (Smith and Riddle, 2009). Permanent infrastructure includes living quarters, scientific laboratories, and engineering and maintenance facilities supporting station operations. Disposal of untreated macerated sewage and wastewater at Davis Station is achieved via a single system with discharge into the sea. Sewage and wastewater are collected into a holding tank in each of eight serviced buildings. On reaching a fixed volume the contents of the tanks are pumped into a wastewater line for discharge. Between April and November the discharge is deposited onto or through sea ice. Between December and March discharge is