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Imported anthropogenic bacteria may survive the Antarctic winter and introduce new genes into local bacterial communities

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Abstract: We studied dynamic changes in anthropogenic bacterial communities at a summer-operated Czech research base (the *Mendel* Research Station) in the Antarctic during 2012 and 2013. We observed an increase in total numbers of detected bacteria between the beginning and the end of each stay in the Antarctic. In the first series of samples, bacteria of *Bacillus* sp. predominated. Surprisingly, high numbers of Gram-positive cocci and coliforms were found (including opportunistic human pathogens), although the conditions for bacterial life were unfavourable (Antarctic winter). In the second series of samples, coliforms and Gram-positive cocci predominated. Dangerous human pathogens were also detected. *Yersinia enterocolitica* was identified as serotype O:9. Antibiotic susceptibility testing showed medium-to-high resistance rates to ampicillin, cefalotin, cefuroxime, amoxicillin-clavulanate and gentamicin in Enterobacteriaceae. 16S rRNA sequencing showed high rates of accordance between nucleotide sequences among the tested strains. Three conclusions were drawn: (1) Number of anthropogenic bacteria were able to survive the harsh conditions of the Antarctic winter (inside and outside the polar station). Under certain circumstances (*e.g.* impaired immunity), the surviving bacteria might pose a health risk to the participants of future expeditions or to other visitors to the base. (2) The bacteria released into the outer environment might have impacts on local ecosystems. (3) New characteristics (*e.g.* resistance to antibiotics) may be introduced into local bacterial communities.

Key words: Antarctic, *Mendel* Station, antropophilic bacteria, biological invasions, spaceflight medicine.

Introduction

Antarctic bacterial communities are composed of psychrophilic and psychrotolerant microorganisms. In certain areas of the Antarctic, extensive research has been performed to determine the principles of the functional ecology of local ecosystems, including microbial ones (*e.g.* Chan *et al.* 2013). A number of bacteria that are common in the Antarctic, can also be found in other places on Earth (*i.e.* are ubiquitous), *e.g. Pseudomonas* sp. (Carrión *et al.* 2011; Michaud *et al.* 2012). Moreover, a large number of bacterial species are endemic to the Antarctic and it is probable that many new species will be discovered (*e.g.* Peeters *et al.* 2011; Kosina *et al.* 2013). These species may carry new attributes, *e.g.* the production of metabolites with antibacterial effects (Rojas *et al.* 2009). It can be presumed that human presence can affect these special ecosystems.

Current knowledge of anthropogenic biological invasions of the Antarctic concerns mainly the introduction of multicellular organisms. The problem is widely discussed in the paper by Frenot *et al.* (2005). However, invasions also occur at the microscopic level, since non-indigenous viruses, bacteria and fungi are introduced into the polar regions (Cowan *et al*. 2011). The potential sources of human-borne bacterial species introduced to the Antarctic include tourism, fishing boats, supply ships, and aeroplanes, as well as the contribution from Antarctic research stations (Frenot *et al.* 2005).

The risk of acquiring an anthropogenic infection by Antarctic animals has been intensively studied during the last few decades. Several studies have identified the presence of human-imported bacteria in Antarctic and subantarctic wild animals. Olsen *et al.* (1996) detected *Salmonella enteritidis* phage type 4 in the faeces of a gentoo penguin on South Georgia. Palmgren *et al*. (2000) reported significant increases in the numbers of penguins and fur seals on South Georgia infected by *S. typhimurium*, *S. havana*, and *S. enteritidis* in 1998 compared to data from 1996. Broman *et al.* (2000) reported the presence of *Campylobacter jejuni* in cloacas of three macaroni penguins on Bird Island, South Georgia. Following this work, Griekspoor *et al.* (2009) performed genetic tests on the three isolated bacterial strains using MLST (multilocus sequence typing). The authors concluded that all three isolated strains genetically matched the strains isolated frequently in human gastroenteritis. Curry *et al*. (2002) focused on the possibility of the transmission of bacteria to colonies of Antarctic animals via tourist boots and concluded that this mode of transmission is possible. García-Peña *et al*. (2010) demonstrated the presence of *C. lari* in the gut of Antarctic fur seals on Deception Island. By contrast, Bonnedahl *et al.* (2005) found no *C. jejuni* or *Salmonella* spp. or *Yersinia* spp. in the six penguin colonies studied in the Antarctic.

Further studies have demonstrated indirect signs of contact between Antarctic animals and imported microbes. For example, Thomazelli *et al.* (2012) reported the finding of Newcastle disease virus in two penguins and the presence of anti-

bodies in the serum of thirty-three others from a sample of a hundred penguins examined on King George Island.

To date, the two best documented ways of introducing non-native bacteria to the Antarctic are aerial transport and the release of bacteria from Antarctic research stations into seawater.

It is postulated that air currents in higher levels of the atmosphere may act as vectors of bacterial transmission for very long distances. The usual routes of bacterial transmission by air and the possible impacts on Antarctic ecosystems are discussed by Pearce *et al.* (2009). Bottos *et al.* (2003) found a number of bacteria commonly recorded on other continents in the air above the Antarctic McMurdo Dry Valleys. Hughes *et al.* (2004) demonstrated the presence of bacteria originating from remote areas of Antarctica in the air above the British *Rothera* Station. Pearce *et al*. (2010) isolated bacteria regularly found in areas inhabited by humans in the air above the British *Halley* Base.

A number of studies have been performed to evaluate the anthropogenic bacterial contamination of seawater in the vicinity of Antarctic research stations. Most studies refer to the release of faecal coliforms and enteric bacteria into the seawater, *e.g.* near the U.S. *McMurdo* Station (Lisle *et al.* 2004), along with the findings of *Escherichia coli* near the British *Faraday* Station (Harker 1989) and *E. coli* and *Clostridium perfringens* near *Rothera* Station (Hughes 2003). Anthropogenic bacteria were found in seawater at distances of up to several hundreds of meters from *McMurdo* Station (McFeters *et al.* 1993) and *Rothera* Station (Hughes and Thompson 2004), and even up to 2 km from the French *Port-aux-Français* Station in the subantarctic Iles Kerguelen archipelago (Delille and Gleizon 2003). Interestingly, Hughes and Blenkharn (2003) referred to an effective method of reducing the bacterial load released from sewage into the seawater (the continuous flushing of sewage-containing tanks by seawater).

There is a total lack of evidence as to how bacterial communities change during periods of human presence or absence at seasonal-operated research stations. This became the subject of our work over a 2-year period between 2012 and 2013. The survival of imported bacterial species and changes in bacterial communities at *Mendel* Station (James Ross Island, the Antarctic) were monitored. We focused on the detection of anthropophilic bacteria with a potential clinical or environmental impact. Changes during the Antarctic winter were also monitored.

We hypothesized that the vast majority of anthropophilic bacteria would not survive the harsh conditions at the base during the winter period. We presumed that only a small number of sporulating Gram-positive rods and possibly a few species of coliforms might be isolated after the 10-month periods of human absence.

Study area

Mendel Station is a summer-operated (January to March) base situated on James Ross Island, ca 20 km east of the Antarctic Peninsula (Fig. 1). The station

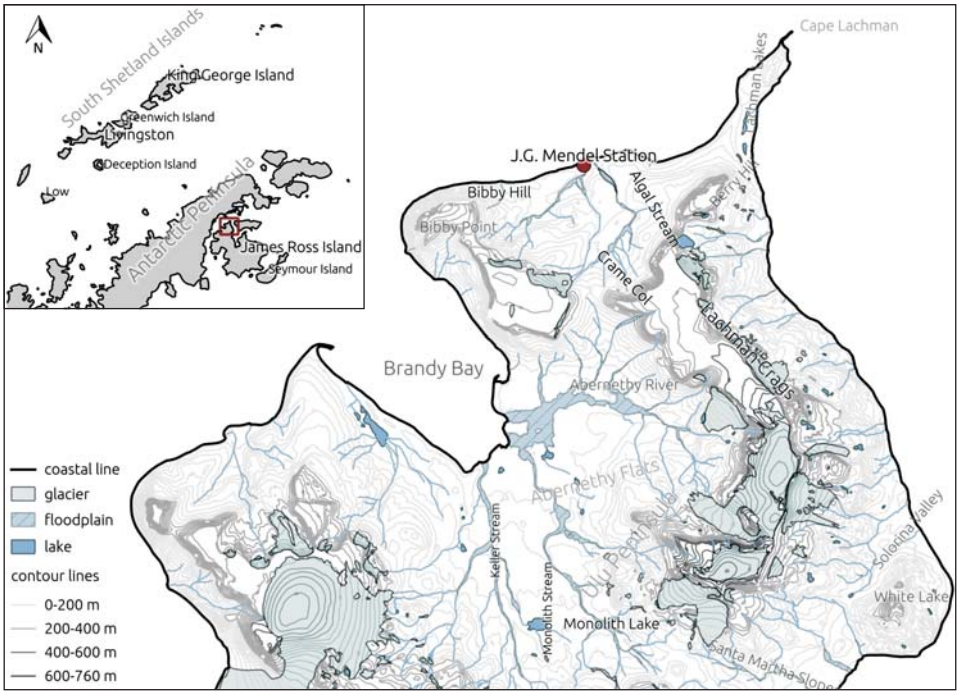

Fig. 1. Study area: northern part of James Ross Island (the Antarctic) with location of *Mendel* Station.

has been operated by Masaryk University since January 2007. The coordinates of the base are 63°4826.5”S, 57°5327.9”W; its elevation is 9 m.a.s.l. The capacity is 20 persons; the facilities include electric power, hot and cold water and air conditioning. Waste water is collected in a cesspool, from where it is drained directly into the sea (without disinfection or filtration). The temperature inside the main building ranges from 16 to 22°C. During the period of human absence, the temperature inside the base can decrease to -17°C. Fig. 2 presents interior base temperatures and outside temperatures recorded during the years 2012 and 2013.

Czech Antarctic Scientific Expeditions (CASE) have been held since 2007 and the average number of participants is 16 persons. An icebreaker of the Chilean navy or aircraft and helicopters of the Argentine army are usually used for the transport of scientific cargo, food and the participants of the expedition. Perfect health is a necessary precondition for participation in the expedition.

There are no permanent penguin or marine mammal colonies in the vicinity of *Mendel* Station, but wild animals occasionally occur during the austral summer. However, no sightings of penguins or marine mammals were made by the 7th CASE during the 2013 austral summer season; this was due to the anomalous quantity of sea ice that remained until the base was abandoned (February 16th 2013). In the proximity of the base, only a few nests of breeding south polar skuas (*Stercorarius maccormicki*) could be found.

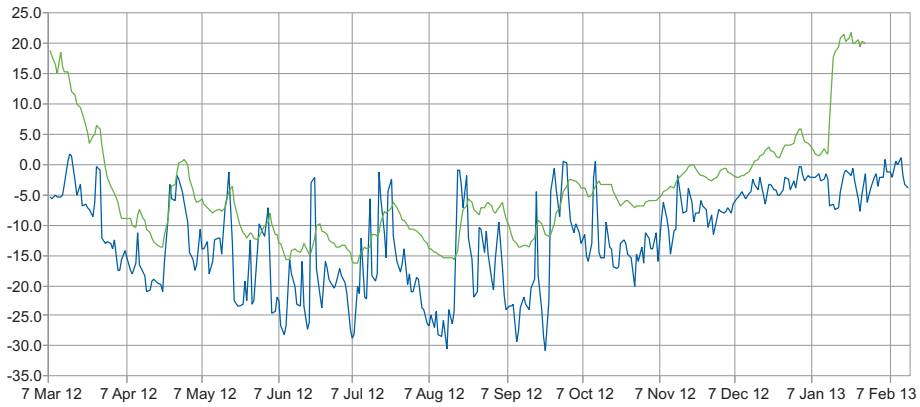

Fig. 2. Annual temperature (°C) recorded at *Mende*l Station, James Ross Island, Antarctica (March 7th, 2012 – February 13th, 2013): grey line: indoor temperatures (*Mendel* Station); dark line: outdoor temperatures (recorded in a distance of *ca* 50 m from the station).

Materials and methods

Sample collection. — Two hundred and four smears were performed at *Mendel* Station during the 6th and 7th CASE – half of them at the beginning of the stay in Antarctica and half one day pre-departure. The first sets of smears were performed immediately after the opening of the base (on January 28th 2012 and January 13th 2013). The person who collected the samples was the very first person to enter the base after 10 months of human absence. No other person was allowed to enter the building until the smears were completed. The second sets were collected one day pre-departure (March 8th 2012 and February 16th 2013) from the same sites as the smears of the first sets.

The smears were taken from twenty-six different sites mostly inside the base (all rooms, including dormitories, the kitchen, the cloakroom, laboratories, storerooms, toilets, and showers, as well as washbasins and the interior and external terminals of the air conditioning system) from 100 cm^2 of the respective surface using sterile swabs. Several smears were also performed outside the main building (the septic tank, the sewage outfall pipe, and a sample of soil taken 20 meters from the sewage outfall pipe).

One half of the swabs were placed into Amies transport medium (Copan Diagnostics Inc., USA) and stored at room temperature. Bacteria from the second half of the swabs were transferred to the sterile globules of ITEST KRYOBANKA B test tubes (ITEST plus, Czech Republic) used for long-term preservation. The test tubes were stored at -18°C.

Bacteria cultivation and identification. — At the laboratory in the Czech Republic, the swabs and the globules were inoculated into a culture broth (Nutrient Broth, HiMedia, India) and then cultivated for 48 hours at 37°C. Subsequently, the culture was inoculated onto Columbia Agar (Merck, Germany), MacConkey Agar

(Conda, Spain), and chromogenic agar UriSelect 4 (BioRad, France) and cultivated for between 24 and 48 hours at 37°C.

The phenotypic characteristics of the isolates were determined using commercial identification systems: MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) microflexTM LT with IVD MALDI Biotyper 2.2 software (Bruker Daltonics, Germany) and Biolog®system (Garland and Mills 1991). Identification was performed according to the manufacturer's instructions. The MALDI Biotyper identifies microorganisms using MALDI-TOF Mass Spectrometry to detect a unique protein fingerprint which is specific down to species level. The Biolog® system is based on the utilization of a suite of 95 different carbon sources, which allows the rapid and reliable identification of bacteria and also the determination of novel taxa (*e.g.* Sedláček *et al.* 2012; Pantůček *et al.* 2013). Microplates for the identification of Gram-negative (Biolog GN2 Micro Plate), as well as gram-positive bacteria (Biolog GP2 Micro Plate) were used.

Antibiotic susceptibility assay. — Antibiotic susceptibility was tested in Enterobacteriaceae and *Enterococcus* sp. using the disc diffusion method according to http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf. Briefly, the culture was diluted in sterile saline to an OD_{600} of 0.5. The suspension was diluted 100× and plated on the Mueller-Hinton Agar (HiMedia, India). The set of discs with antibiotics included 10 µg ampicillin, 30 µg cefalotin, 30 µg cefuroxime, 5 µg ciprofloxacin, 25 µg trimethoprim/sulfamethoxazole, 10 µg norfloxacin, 10 µg gentamicin, 30 µg cefotaxime, 30 µg ceftazidime, 30 µg amoxicillin/clavulanic acid, 30 µg aztreonam, 30 µg chloramphenicol, and 10 µg colistin for Enterobacteriaceae; and 10 µg ampicillin, 10 µg gentamicin, 5 µg vancomycin, 15 µg quinupristin/dalfopristin, 30 µg chloramphenicol, 15 µg tigecycline, 10 µg linezolid for *Enterococcus* sp. (all antibiotic samples from Oxoid, United Kingdom). The plates were incubated at 35°C for 20 h. The inhibition zones were measured subsequently and compared with the zone diameter interpretative criteria for the used antibiotics. *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 20212 were used as quality controls.

Serotype classification. — *Yersinia enterocolitica* was serotyped using a previously described method (Aleksić and Bockemühl 1984) with diagnostic agglutination antisera O:3, O:5, O:8 and O:9 (ITEST PLUS, Czech Republic). Strains *Y. enterocolitica* YE18, YE63, YE110, YE17 (Bosák *et al.* 2013) were used as positive controls.

16S rRNA sequencing. — This method was performed in those species that were detected in all series of the collected samples. These included *Klebsiella oxytoca* and *Pantoea agglomerans*.

Colony PCR was performed for amplification of the 16S rRNA gene; universal primers 27F: 5´-AGA GTT TGA TCC TGG CTC AG-3´ (Lane 1991) and 1541R: 5´-AAG GAG GTG ATC CAG CCG CA-3´ (Zhou *et al.* 1997) were used. The

PCR products were purified using QIAquick PCR Purification Kit (QIAGEN, Venlo, Netherlands) and sequencing was performed by Eurofins MWG Operons (Ebersberg, Germany). Partial sequences of the 16S rRNA gene were assembled using Geneious software (Kearse *et al*. 2012).

Results

A total of 469 isolates were acquired. Of these, 310 isolates (66.09%) were identified to species level and 88 isolates (18.77%) to genus level.

In the first series of samples, Gram-positive spore-forming rods (*Bacillus* sp., *Paenibacillus* sp., and *Brevibacillus* sp.) predominated. A wide range of opportunistic pathogens were also detected, including Gram-positive cocci (*Enterococcus* sp., *Staphylococcus* sp.) and coliforms (*Citrobacter* sp., *Enterobacter* sp., *Serratia* sp., *Escherichia coli*, *Yersinia enterocolitica*). We underline that these bacteria were isolated after ca 10 months of human absence at the polar base.

At the end of each season, we observed an increase in the number of detected species and also in the total number of isolates. The bacterial communities shifted towards the dominance of coliforms and Gram-positive cocci, including human pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*).

In the first series of samples from 2012, a total of 86 bacterial strains were isolated. Of these, 45 isolates were identified to species level (26 species of bacteria) and 26 isolates to genus level (11 genera). The most frequent species were *Bacillus pumilus* (isolated 7×), *Bacillus megaterium* (4×), and *Aerococcus viridans* (4×). The most abundant genus was *Bacillus*, which made up 37.2% of all isolated strains. Opportunistic pathogens including, for example, *Staphylococcus hominis*, *Staphylococcus xylosus*, *Stenotrophomonas maltophilia* and a strain of the genus *Clostridium* were isolated. Interestingly, *Yersinia enterocolitica* and *Serratia fonticola*, together with 2 strains of the genus *Aeromonas*, were isolated from the septic tank (located outside the main building). In the soil near the sewage outfall pipe, one strain of *Enterococcus faecium* was isolated.

From the swabs collected at the end of the 6th CASE, a total of 117 bacterial strains were isolated. Of these, 77 isolates were identified to species level (32 species) and 22 isolates to genus level (9 genera). The most frequent species were *Klebsiella oxytoca* (11×), *Citrobacter freundii* (9×), *Enterobacter cloacae*, and *Stenotrophomonas maltophilia* (both 6×), while the most abundant genus was *Staphylococcus* (12.0% of all isolated strains). Opportunistic pathogens from this series included, for example, *Enterobacter cloacae*, *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus hominis*, *S. epidermidis*, *Citrobacter freundii*, *Stenotrophomonas maltophilia,* and *Salmonella* sp. A large number of viable bacteria were isolated from the septic tank and from seawater, including, for example, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella oxytoca*, and *Citrobacter freundii*.

From the swabs collected at the beginning of the 7th CASE, a total of 111 bacterial strains were isolated. Seventy-one isolates were identified to species level (38 species) and 20 isolates to genus level (9 genera). *Pantoea agglomerans* (8×), *Bacillus subtilis* and *Klebsiella oxytoca* (both 5×) were the most frequent bacteria, while *Bacillus* was the most frequent genus (17.1% of isolates). Opportunistic pathogens included *Enterococcus faecalis*, *Enterobacter cloacae*, *Escherichia coli* and staphylococci (*Staphylococcus hominis*, *S. epidermidis*, *S. warneri*).

At the end of the 7th CASE, a total of 155 bacterial strains were isolated, of which 117 were identified to species level (60 species) and 20 isolates to genus level (6 genera). The most frequent species were *Acinetobacter pittii*, *Klebsiella oxytoca* (both 10×) and *Enterococcus faecalis* (6×). The most abundant genus was *Staphylococcus* (14.8%). Opportunistic pathogens included *Staphylococcus aureus* and other staphylococci (*S. hominis, S. epidermidis, S. haemolyticus, S. warneri*), *Pseudomonas aeruginosa*, *Enterococcus faecalis, Klebsiella pneumoniae* and other Enterobacteriaceae. In addition, S*treptococcus uberis* and a further unspecified strain of *Streptococcus* sp. were isolated.

A list of pathogens with potentially highest clinical relevance is presented in Table 1, while lists of bacterial families represented in each series of samples are presented in Table 2. Changes in the structures of bacterial communities at *Mendel* Station are presented in Fig. 3. Complete lists of the identified bacteria are shown in Supplementary Online Material (tables 1–4) available at http://www.degruyter.com/ view/j/popore.2016.37.issue-1/ popore-2016-0001/ suppl/popore-2016-0001_suppl.pdf.

The *Y. enterocolitica* strain (isolated from the septic tank in 2012 after the winter period) was identified as serotype O:9, which is a common clinical isolate in Europe and North America.

Antibiotic susceptibility testing showed medium-to-high rates of resistance (16–72%) to ampicillin, cefalotin, cefuroxime, amoxicillin-clavulanate, and gentamicin in Enterobacteriaceae. Resistance rates were low in enterococci, except for quinupristin-dalfopristin (resistence rate 50%). Resistance rates are presented in Fig. 4.

16S rRNA sequencing showed that the 6 tested *Klebsiella oxytoca* strains matched in 99.01% of their nucleotides, differing only in 14 out of a total of 1412 (0.99%). Similarly, the 4 tested *Pantoea agglomerans* strains showed an overall match of 98.8% with respect to their 1125 nucleotides.

Discussion

To the best of our knowledge, this is the first report of changes in the bacterial environment of a summer-operated Antarctic research station over a period of two years. The impact of human activities on microbial commmunities in the vicinity of permanently inhabited Antarctic research stations has been the subject of a number of studies. Boyd and Boyd (1963) reported a finding of *Escherichia coli* in

Table 1

A list of the most pathogenic bacteria isolated from all samples: I /2012: first series of smears in 2012; II/2012: second series of smears in 2012; I/2013: first series of smears in 2013; II/2013: second series of smears in 2013.

I / 2012	II / 2012	I / 2013	II / 2013
Enterobacter amnigenus	*Acinetobacter johnsonii*	*Acinetobacter lwoffii*	*Citrobacter freundii*
Enterococcus faecium	*Citrobacter freundii*	*Enterobacter amnigenus*	*Enterobacter cloacae*
Pantoea agglomerans	*Enterobacter amnigenus*	*Enterobacter cloacae*	*Enterococcus faecalis*
Staphylococcus hominis	*Enterobacter cloacae*	*Enterobacter avium*	*Escherichia coli*
Staphylococcus xylosus	*Enterococcus faecium*	*Enterococcus faecalis*	*Klebsiella pneumoniae*
Stenotrophomonas maltophilia	*Escherichia coli*	*Escherichia coli*	*Pseudomonas aeruginosa*
Yersinia enterocolitica	*Klebsiella oxytoca*	*Klebsiella oxytoca*	*Staphylococcus aureus*
Clostridium sp.	*Pseudomonas aeruginosa*	*Pantoea agglomerans*	*Staphylococcus epidermidis*
Salmonella sp.	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus hominis*
	Staphylococcus hominis	*Staphylococcus hominis*	*Stenotrophomonas maltophilia*
	Salmonella sp.	*Staphylococcus warneri*	*Streptococcus* sp.

Table 2

The numbers of bacterial species in particular recorded families detected at *Mendel* Station over 2 years: I/2012: first series of smears in 2012; II/2012: second series of smears in 2012; I/2013: first series of smears in 2013; II/2013: second series of smears in 2013.

Family / Period	I/2012	II/2012	I/2013	II/2013
Aerococcaceae	5	0	0	0
Alcaligenaceae	1	0	0	2
Bacillaceae	32	14	28	14
Caulobacteraceae	0	0	0	1
Clostridiaceae	1	1	1	1
Comamonadaceae	1	2	1	0
Corynebacteriaceae	0	0	2	0
Enterobacteriaceae	11	49	28	43
Enterococcaceae	1	3	4	9
Leuconostocaceae	0	0	2	1
Microbacteriaceae	0	0	2	0
Micrococcaceae	2	0	7	2
Moraxellaceae	0	2	2	20
Pseudomonadeaceae	9	8	5	12
Sphingobacteriaceae	0	0	1	1
Staphylococcaceae	5	14	7	28
Streptococcaceae	0	0	1	4
Xanthomonadaceae	0	6	0	0

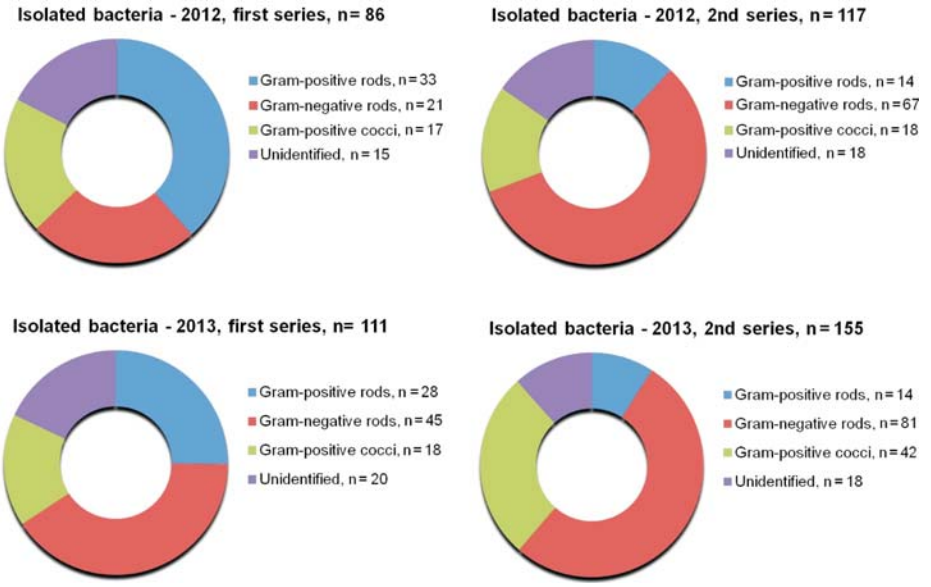

Fig. 3. Overall changes in composition of bacterial communities (differentiated by Gram staining) isolated during the years 2012 and 2013 from *Mendel* Station. First series of samples were collected after ca 10 months of human absence at the station (*i.e.* after the Antarctic winter), the second series after 2 months of human presence at the base (*i.e.* before the station was closed).

the soil at the garbage dump of Shackleton's expedition. It is questionable whether the single isolated *E. coli* was really a strain surviving from 1909. Xiao *et al.* (2007) found *Bacillus* sp. and *E. coli* in the vicinity of the Chinese *Great Wall* Station. Hughes and Nobbs (2004) isolated a number of viable bacteria (*Bacillus* sp., *Micrococcus* sp., *Aerococcus* sp., *Streptococcus* sp. and *Clostridium perfringens*) from samples taken from a 30- to 40-year-old garbage deposit at Fossil Bluff.

In our study, we found the survival of *Enterococcus faecium* and *Yersinia enterocolitica* during the winter period, and of *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella oxytoca* and *Citrobacter freundii* during the period of human presence in the vicinity of *Mendel* Station, *i.e.* in the outer environment. These bacteria survived unfavourable living conditions during the Antarctic winter, which included a total lack of nutrients and water. Our most important finding was the survival of viable strains of *Yersinia enterocolitica* and *Enterococcus faecium* during winter in the vicinity of *Mendel* Station. *Y. enterocolitica* serotype O:9 is a common clinical isolate in Europe (Fukushima *et al.* 1998; Rosner *et al.* 2010). To date, the presence or survival of *Y. enterocolitica* in an Antarctic environment has never been reported. However, in a study by Smith *et al.* (1994), 4 species of pathogenic bacteria (including *Y. enterocolitica*) were exposed to conditions imitating those in the Southern Ocean in the vicinity of *McMurdo* Station. A large number of the exposed bacteria were able to survive the extreme conditions, but only a minimal number were able to create colonies on solid media. Similarly, there have been no

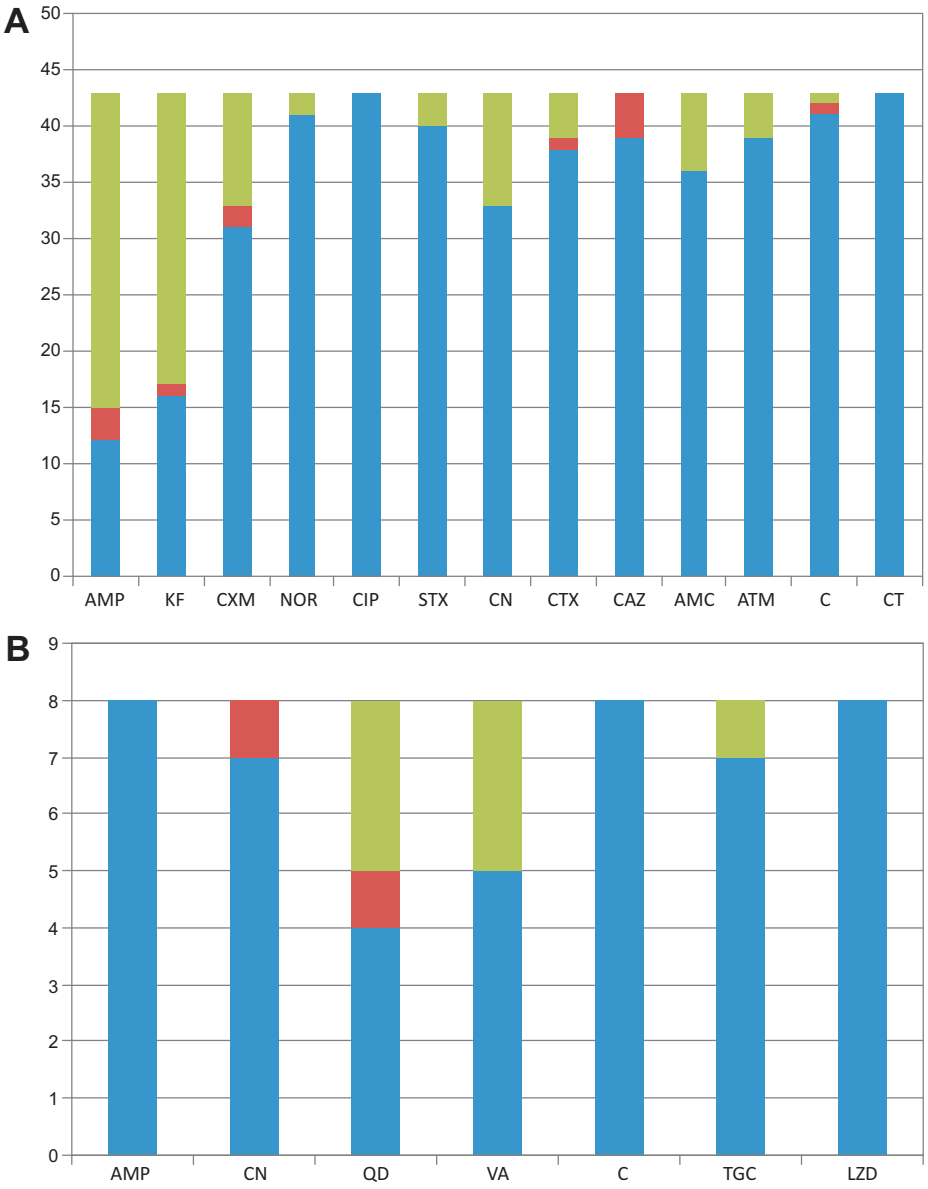

Fig. 4. **A**. Susceptibility to antibiotics in family Enterobacteriaceae: blue – strains susceptible to the tested antibiotic, red – intermediate resistance; green – resistant strains x-axis: antibiotics: AMP – ampicillin, KF – cefalotin, CXM – cefuroxime, NOR – norfloxacin, CIP – ciprofloxacin, STX – trimethoprim/sulfamethoxazole, CN – gentamicin, CTX – cefotaxime, CAZ – ceftazidime, AMC – amoxicillin/clavulanic acid, ATM – aztreonam, C – chloramphenicol, CT – colistin; y-axis: number of tested bacterial strains (family Enterobacteriaceae). **B**. Susceptibility to antibiotics in *Enterococcus* sp.: blue – strains susceptible to the tested antibiotic, red – intermediate resistance; green – resistant strains x-axis: antibiotics: AMP – ampicillin, CN – gentamicin, QD – quinupristin/dalfopristin, VA – vancomycin, C – chloramphenicol, TGC – tigecycline, LZD – linezolid; y-axis: number of tested bacterial strains (*Enterococcus* sp.).

reports of *Klebsiella oxytoca* or *Pantoea agglomerans* surviving in the Antarctic environment. *E. faecium* strains were found in seawater in the vicinity of a few research stations (Delille and Gleizon 2003; Lisle *et al*. 2004), but never on land.

We also isolated a large number of non-native bacteria that survived the winter period inside the main building of *Mendel* Station, including staphylococci, *Enterococcus faecalis*, *Enterobacter cloacae* and *Escherichia coli*. Under certain conditions, these bacteria might become a source of infectious disease for the participants of forthcoming expeditions. Interhuman transfer of bacteria among crew members of Antarctic expeditions was studied during long term stays of isolated groups of participants. Cameron (1970) demonstrated increased rates of colonisation by staphylococci in expedition participants at *Mawson* Station during the winter period. Krikler (1986) demonstrated the interhuman transmision of certain strains of *Staphylococcus aureus* between participants of the expedition wintering at *Halley* Station. Tzabar and Pennington (1991) demonstrated the transfer of *Escherichia coli* between crew members during their stay at *Signy* Base. Van Houdt (2009) examined the presence of bacteria in the air at *Concordia* Base during a one-year stay. The most frequently isolated bacteria were *Staphylococcus* sp. and *Bacillus* sp. To date, however, there are no reports of severe bacterial infections in humans during stays at polar research stations in the Antarctic.

Anthropophilic bacteria may carry and transfer new attributes, *e.g.* resistance to antibiotics. We demostrated medium to high resistance rates to ampicillin, cefalotin, cefuroxime, amoxicillin-clavulanate, and gentamicin among enterobacteria. Miller *et al.* (2009) studied bacterial resistance to antibiotics at the U.S. *Palmer* Station. The resistance rate was higher among mesophilic bacteria when compared to psychrophiles and the rate of resistance decreased with increasing distance from the base. Hernandez *et al.* (2012) found ESBL (extended-spectrum beta-lactamase) strains of *Escherichia coli* in seawater in the vicinity of the Chilean Antarctic stations *Arturo Prat* and *Bernardo O'Higgins*. Timmery *et al*. (2011) investigated resistance to antibiotics, toxin production, and the ability to transfer plasmids in isolates of *Bacillus* sp. from *Concordia* base and the International Space Station (ISS). The authors found low virulence, but a high potential for plasmid transfer. Schiwon *et al*. (2013) found a higher frequency of resistance to antibiotics, transfer genes, and plasmid-bearing strains in *Staphylococcus* and *Enterococcus* spp. from *Concordia* base when compared to the strains isolated from the ISS station. Moreover, the transmission of resistance genes from staphylococci to *E. faecalis* and *S. aureus* was demonstrated. In contrast, in a study of bacteria isolated from various glaciers in different continents including Antarctica and Greenland, Segawa *et al*. (2013) found that bacteria from all regions of the world carried antibiotic resistance genes with the exception of bacteria isolated from Antarctic glaciers.

In many respects, the Antarctic represents an appropriate environment for studying the possible risks posed by microorganisms in human spaceflight. If bacteria are able to survive long-term exposure to extreme conditions or even acquire new attrib-

utes, this could pose a severe problem during long-duration spaceflight (*e.g.* the Mars project). In a study by Novikova *et al.* (2006), the colonisation of the ISS by a large number of bacterial species was demonstrated. The severity of this phenomenon could be amplified by changes to the human immune system that have been demonstrated in several physiological studies. For example, the effects of long-term isolation on the human immune system was studied by Shirai *et al.* (2003) and by physicians participating in Australian Antarctic expeditions in the 1980s and 1990s (Lugg and Shepanek 1999). Long-term deprivation of the immune system (due to a relative lack of stimuli) leads to a gradual decrease in the effectiveness of certain immunological mechanisms. An overview of current knowledge on spaceflight-associated immunological changes and health risks is presented in Guéguinou *et al.* (2009).

Conclusions

We conclude that a large number of mesophilic bacteria can survive a period of human absence of approximately 10 months. Besides the expected survival of sporeforming bacterial species, we detected the survival of a number of opportunistic human pathogens. This finding has three major potential implications. First, the surviving anthropogenic bacteria were able to withstand the harsh conditions of the Antarctic winter (inside and outside the polar station). Under certain circumstances (*e.g.* impaired immunity), the surviving bacteria might pose a health risk to the participants of future expeditions (or to other visitors to the base). Second, the bacteria released into the external environment might have potential impacts on local ecosystems – for example, by causing infection in animals. Third, anthropophilic bacteria released into the Antarctic environment may introduce new characteristics (*e.g.* resistence to antibiotics) into the local bacterial communities.

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