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Comparison of Antibiotic Resistance, Biofilm Formation and Conjugative Transfer of *Staphylococcus* and *Enterococcus* Isolates from International Space Station and Antarctic Research Station Concordia

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Abstract The International Space Station (ISS) and the Antarctic Research Station Concordia are confined and isolated habitats in extreme and hostile environments. The human and habitat microflora can alter due to the special environmental conditions resulting in microbial contamination and health risk for the crew. In this study, 29 isolates from the ISS and 55 from the Antarctic Research Station Concordia belonging to the genera *Staphylococcus* and *Enterococcus* were investigated. Resistance to one or more antibiotics was detected in 75.8 % of the ISS and in 43.6 % of the Concordia strains. The corresponding resistance genes were identified by polymerase chain reaction in 86 % of the resistant ISS strains and in 18.2 % of the resistant Concordia strains. Plasmids are present in 86.2 % of the ISS and in 78.2 % of the Concordia strains. Eight *Enterococcus faecalis* strains (ISS) harbor plasmids of about 130 kb. Relaxase and/or transfer genes encoded on plasmids from gram-positive bacteria like pIP501, pRE25, pSK41, pGO1 and pT181 were detected in 86.2 % of the ISS and in 52.7 % of the Concordia strains. Most pSK41-homologous transfer genes were detected in ISS isolates belonging to coagulase-negative staphylococci. We demonstrated through mating experiments that *Staphylococcus haemolyticus* F2 (ISS) and the Concordia strain *Staphylococcus hominis* subsp. *hominis* G2 can transfer resistance genes to *E. faecalis* and *Staphylococcus aureus,* respectively. Biofilm formation was observed in 83 % of the ISS and in 92.7 % of the Concordia strains. In conclusion, the ISS isolates were shown to encode more resistance genes and possess a higher gene transfer capacity due to the presence of three *vir* signature genes, *virB1*, *virB4* and *virD4* than the Concordia isolates.

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Introduction

Microorganisms are ubiquitously present in nature and have the ability to survive and to prosper under variable and wide

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ranges of environmental conditions. Closed environments like the ISS and the Antarctic Research Station Concordia provide a very special environmental niche for microorganisms with direct or indirect impact on health, safety or performance of the crew. The crew is, along with the residing environmental microorganisms, the most important contamination source of closed environments. Microorganisms are present on the human skin, mucous membranes, upper respiratory tract, mouth and gastrointestinal tract. They can be spread through numerous exposure routes (e.g. air, personal contact, etc.).

The airborne contamination in Concordia is dominated by Bacilli and α-Proteobacteria. *Staphylococcus* and *Bacillus* species were the predominant members of the Bacilli class. They also proved to be the most frequently isolated bacteria from surface and air samples aboard the ISS. Both genera belong to the normal human microflora [1–3]. This prevalence is in accordance with multiple other studies from indoor air, hospital wards, apartments, multi-family buildings, homes and offices [4–6].

Microorganisms are commonly found on surfaces of materials and hardware of the spacecraft. Biofilm growth was observed on rubber seals, viewing windows and different hardware surfaces. Biofilms may cause damage to the equipment, which can lead to severe technical problems [7]. In the Mir space station, biofilms damaged quartz windows, corroded various metals and caused polymer deterioration [8, 9].

For a range of microbial species, enhanced virulence, increased antibiotic resistance and differential gene expression under spaceflight conditions have been reported, which can have important potential impacts on human health [summarised in 2, 10]. Additionally, bacteria under spaceflight conditions showed enhanced secondary metabolite and extracellular polysaccharide production as well as biofilm formation [9, 11]. These bacteria may spread their virulence and/or antibiotic resistance genes through horizontal gene transfer (HGT) and turn harmless bacteria into potential pathogens [12, 13].

HGT is mediated by mobile genetic elements (MGEs), such as plasmids, conjugative transposons, integron-specific gene cassettes or phages that are able to facilitate their own transfer. They are involved in the dissemination of important traits such as antibiotic resistance, virulence determinants, production of toxins or the ability to exploit specific niches. Especially plasmid-mediated HGT plays an important role in the emergence of new pathogens [12]. Plasmid-encoded conjugative signature genes are *virD2*-like relaxases and the *virB1* (lytic transglycosylase), *virB4* (ATPase) and *virD4* (coupling protein) homologs of the prototype *Agrobacterium tumefaciens* T-DNA transfer system.

Staphylococci and enterococci are a part of the normal human flora and thus are commensal microorganisms. However, they can also be opportunistic pathogens that cause a wide range of diseases. MGEs are important in the acquisition and dissemination of antibiotic resistance, and staphylococci and enterococci serve as reservoirs and turntables in spreading mobile resistance determinants. Resistance, especially, to multiple antibiotics encoded on plasmids, which can serve as vehicles for transposons and integrons, is prevalent in a variety of pathogenic bacteria including vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, enterococcal conjugative plasmids have been shown to transfer vancomycin resistance to MRSA resulting in vancomycin-resistant *S. aureus* [14–16].

The best characterised broad-host-range plasmids in enterococci are the incompatibility (Inc) group 18 plasmids, pIP501, pAMβ1 and pRE25. They encode resistance to the macrolide-lincosamide-streptogramin group of antibiotics. pIP501 encodes additionally resistance to chloramphenicol, and pRE25 to kanamycin, streptomycin and streptothricin. These plasmids are able to spread their antibiotic resistances to a wide variety of gram-positive (G+) bacteria. pIP501 can also self-transfer to the gram-negative (G−) bacterium *Escherichia coli* [15, 17–19]. pRUM (24.8 kb) is common in *Enterococcus faecium* strains and encodes resistance to erythromycin, chloramphenicol, streptomycin and streptothricin [15].

Conjugative multiresistance plasmids were also found in staphylococci and are exemplified by the pSK41 family. This family includes the plasmids pSK41, pGO1, pLW1043, pV030-8, pPR9 and pUSA03. They share the same genetic backbone, but the antibiotic resistance cassettes are extremely diverse. Multiple copies of the insertion sequence IS*257*, which plays a key role in the evolution of pSK41-like plasmids, usually flank the resistance genes of this plasmid family [20–22].

In this study, 84 *Staphylococcus* and *Enterococcus* isolates from two confined environments, namely the ISS and the Antarctic research station Concordia, have been analysed with molecular methods. The microbes from ISS and Concordia have been compared with regard to antibiotic resistance, biofilm formation and conjugative transfer capability.

Methods

Bacterial Strains and Plasmids

Strains and plasmids used as reference/control in polymerase chain reaction (PCR) assays and in mating experiments are listed in Table 1. A total of 84 bacterial ISS (29) and Concordia (55) isolates belonging to the genera *Staphylococcus* and *Enterococcus* were selected from a collection of more than 850 strains obtained from

Table 1 Bacterial strains and plasmids

Strain	Characteristics	Reference or source[a]
Bacillus subtilis		
BD662	pBD90 [*erm*D]; *trpC2, thr-5*	[56]
BD1156	pBD370 [*erm*G]; *leu, mer, hisH*	[57]
E. coli XL10	*tet*O	[70]
Enterobacter cloacae		
DSM46348	*amp*C	DSMZ
E. casseliflavus UC73	*aph(2')-Id, van*C	[71]
Enterococcus gallinarum SF9117	*aph*(2')*-Ic*, *van*C, *erm*B	[71]
E. faecalis		
DS16	*tet*M; pAD1, pAD2	[72]
RE25	pRE25 [*erm*B, cat_{pIP501}, *aph(3')-III*, *sat4*, *ant(6)-Ia*, tra^+], *tet*M	[19]
V583	*van*B; pTEF1, pTEF2, pTEF3	[73]
JH2-2	Rif^R, Fus^R	[74]
JH2-2	pIP501 [cat_{pIP501}, *erm*B, tra^+]	[75]
OG1X	$Strep^R$, protease-negative	[76]
E. faecium		
9631160-1	pRI1 [mob_{pRI1}]	[77]
SF11770	*aac(6')-Im, aph(2')-Ib, aac(6')-Ii, ant(4')-Ia, ant(6)-Ia, aph(3')-III, erm*B, *sat4, tet*L, *tet*M, *van*A, *van*Z	[71]
DSM17050	*van*A	DSMZ
L. lactis K214	pK214 [*tet*S, cat_{LM}, *mdt(A), str*, mob^+]	[71]
S. aureus		
RN3259	pT181 [*tet*K, pre_{pT181}]	[78]
SK5428	pSK41 [*ant(4')-Ia*, *aac(6')-Ie-aph(2')-Ia*, *ble*, *qacC*, tra^+]	[79]
DSM13661	*mec*A	DSMZ
RN7242	pGO1 [*ant(4')-Ia*, *aac(6')-Ie-aph(2')-Ia*, *ble*, *dfr*A, *qacC*, tra^+]	[20]
RN4220RF	Rif^R, Fus^R	Robert Koch-Institute, Wernigerode, Germany
S. haemolyticus VPS617	*tet*K, *mph(C), erm*C, *msr, bla*Z, *mec*A, *dfr*A, *aph(3')-III, aph(2')-Ia, aac(6')-Ie, ant(6')-IaInorA, sat4*	[71]
Streptococcus pneumoniae T4 500	pC194 [cat_{pC194}]; *tet1 hex4 T4 mal594*	[80]

Kan^R kanamycin Erm^R erythromycin, Rif^R rifampicin, Fus^R fusidic acid, $Strep^R$ streptomycin resistance

[a]DSMZ: German collection of microorganisms and cell cultures, Braunschweig, Germany

sampling campaigns between 2002 and 2006 in the Antarctic base Concordia and in the ISS in scope of the ESA project MISSEX (Microbial ISS gene Exchange) (Table 2) [1, 2]. The 62.5 % (35) of Concordia and 13.8 % (4) of ISS strains were isolated from the body of crew members; the other strains were derived from air samples. The species of the microbial isolates was identified using VITEK 2 Compact (bioMérieux, Brussels, Belgium) or 16S rDNA sequencing [1, 2].

ISS Crew Sampling and Bacteria Isolation

The ISS crew isolates used in this study were isolated from samples taken in-flight from the groin (IJ) and upper arm (IL) and post-flight from the tongue (OD). Sampling was done by swabs impregnated with a salt buffer-mineral oil mixture, and swabs were stored at ambient temperature in the ISS. Upon return, swabs were re-suspended in 2 ml of saline solution (0.9 % NaCl), and subsequently, a 1-ml aliquot of this solution was mixed with 1 ml of 40 % glycerol solution and stored at −80 °C. Samples were thawed, and appropriate dilutions were plated on tryptic soy agar (TSA) containing cycloheximide (300 $\mu g ml^{-1}$) to inhibit fungal growth. Petri dishes were incubated at 37 °C and scored for bacterial colonies after 2 days of incubation. Morphologically different colonies were counted and purified on TSA plates.

Table 2 Bacilli isolates from Antarctic Base Concordia and ISS (as percentage of total number of isolates), divided into bacterial genera (percent of class) and species (percent of genus)

Concordia		ISS	
Bacilli	52.0	Bacilli	86.0
Staphylococcus	55.8	*Staphylococcus*	87.6
S. hominis	42.1	*S. epidermidis*	37.6
S. epidermidis	17.2	*Staphylococcus species*	27.4
Staphylococcus capitis	9.0	*S. hominis*	9.3
Staphylococcus pasteuri	5.5	*S. saprophyticus*	4.4
S. haemolyticus	4.8	*Staphylococcus pulvereri*	4.0
Staphylococcus species	4.8	*S. haemolyticus*	3.5
Staphylococcus cohnii	4.1	*Staphylococcus xylosus*	3.5
Staphylococcus warneri	4.1	*Staphylococcus equorum*	2.7
S. aureus	3.5	*S. aureus*	1.8
Staphylococcus intermedius	1.4	*Staphylococcus auricularis*	1.8
S. auricularis	0.7	*S. lugdunensis*	1.8
Staphylococcus sciuri	0.7	*S. capitis*	1.3
S. xylosus	0.7	*Staphylococcus arlettae*	0.4
		Staphylococcus lentus	0.4
Enterococcus casseliflavus	0.4	*E. faecalis*	4.3

ISS Air Sampling and Bacteria Isolation

Air sampling was done with the US supplied Microbial Air sampler kit and SAS air sampling device (PBI International, Italy). The media plates were incubated at 37 °C and scored for bacterial colonies after 2 days of incubation. Sample collection, incubation, data recording and interpretation were performed by the crew aboard the ISS [1, 3].

Concordia Crew Sampling and Bacteria Isolation

Hand sampling was done by swabbing the palm of the right hand of the crew member with a preservative-impregnated (25 % glycerol) swab. Samples were stored at −80 °C before further processing. Samples were thawed and re-suspended in 20 ml saline solution (0.9 % NaCl) containing 0.01 % Tween 80. Suspensions were incubated for 1 h on a rotatory shaker to allow extraction of the bacteria from the samples. All samples were filtered over a 0.2 μm Biosart®100 filter (Sartorius, Vilvoorde, Belgium). Filters were placed on TSA to allow bacterial growth. Petri dishes were incubated at 37 °C and 28 °C and scored for bacterial colonies after 2 days of incubation. Morphologically different colonies were counted and purified on TSA plates.

Concordia Air Sampling and Bacteria Isolation

Air sampling was done monthly with the portable AirPort MD8 sampler (Sartorius). The filter unit was aseptically opened, and a filter paper was placed on the gelatin membrane filter. Both filters were stored in 5 ml of a preservative solution (0.85 % NaCl and 20 % glycerol) below −50 °C in an outdoor ice cave. Samples were thawed and suspended in 20 ml saline solution containing 0.01 % Tween 80 and incubated 1 h at room temperature on a rotatory shaker to allow extraction of the bacteria from the samples. Suspensions were filtered over a 0.2 μm Biosart®100 filter (Sartorius), and filters were incubated at 28 °C on TSA containing cycloheximide (300 μg ml^{-1}) for 2 and 5 days and scored for bacterial colonies. Morphologically different colonies were counted and purified on TSA plates [2].

Growth Conditions

All strains were grown in brain heart infusion broth (BHI, Conda, Madrid, Spain) at 37 °C with shaking unless otherwise indicated. BHI broth and agar plates were supplemented, when required, with gentamicin (50 $\mu g ml^{-1}$), streptomycin (1,000 $\mu g ml^{-1}$), chloramphenicol (10 μg ml^{-1}), tetracycline (Tet, 10 $\mu g ml^{-1}$), erythromycin (20 μg ml^{-1}), fusidic acid (50 $\mu g ml^{-1}$), rifampicin (200 $\mu g ml^{-1}$) and nisin (100 $ng ml^{-1}$). *Lactococcus lactis* K214 was grown in de Man, Rogosa and Sharp broth (Carl Roth, Karlsruhe, Germany) supplemented with Tet (10 $\mu g ml^{-1}$).

Disc Diffusion Method

Resistance of the isolates to specific antibiotics was determined by the disc diffusion method with the following antibiotic discs (Oxoid, Wesel, Germany) (micrograms): ampicillin (25), chloramphenicol (10 and 20), erythromycin (10 and 30), gentamicin (10), kanamycin (30), oxacillin (5),

streptomycin (25), tetracycline (10) and vancomycin (5 and 30). BHI overnight cultures were grown at 37 °C. The 100 μl of the overnight culture was plated on BHI agar plates, and the antibiotic discs were applied onto the agar surface with a disc dispenser (Oxoid, Wesel, Germany). The plates were incubated for 24 h at 37 °C, and the zones of inhibition were measured.

DNA Extraction

Isolation of plasmid DNA from *Enterococcus faecalis* was performed according to the protocol of Woodford et al. [23] with modifications by Werner et al. [24]. The plasmid DNA profiles of the staphylococci isolates were obtained by a rapid plasmid DNA isolation method according to Birnboim and Doly [25] and Sambrook and Russel [26]. Plasmid extractions from *E. coli* were performed using QIAprep spin miniprep kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction.

Bacterial lysates were prepared from 100 μl overnight cultures, centrifuged, re-suspended in 20 μl lysis buffer (50 mM NaOH, 0.25 % sodium dodecyl sulfate) and boiled for 20 min. Prior to use in PCR, they were diluted 1:10. Genomic DNA was isolated with High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instruction.

S1-Nuclease Macrorestriction

Plasmid content of the *E. faecalis* strains was evaluated by a modified S1 nuclease macrorestriction analysis based on the Barton method [27, 28]. Briefly, genomic DNA was digested with 14 U of S1 nuclease (Takara, Bop Inc., Shiga, Japan) for 15 min at 37 °C and separated on 1 % agarose gel in PFGE (CHEF III apparatus, Bio-Rad, La Jolla, CA). The ramped pulsed times were as follows—5–35 s for 22 h at 14 °C. *Sma*I-digested *S. aureus* NCTC 8325 was used as a size marker on the PFGE gel. Data were analysed, and plasmid sizes were calculated using a Dice coefficient and UPGMA clustering (BioNumerics v. 5.1; Applied Maths, Sint-Martens-Latem, Belgium).

Primer Design

Oligonucleotide primers to amplify relaxase genes of conjugative and mobilisable plasmids from G+ bacteria were selected on basis of conserved regions identified by sequence comparison of selected relaxase genes from the MOB_Q and MOB_V family [29–31].

Oligonucleotide primers for detecting *virB1*, *virB4* and *virD4* homologous genes from pIP501 (*orf7*, *orf5* and *orf10*) and pSK41 (*traG*, *traE*, *traK*) as well as from closely related plasmids were designed on basis of published DNA sequences (GenBank). Additionally, two oligonucleotide primers for the essential transfer genes *traM*/*trsM* and *traL*/*trsL* encoded by the conjugative plasmids pSK41/pGO1 were generated.

The oligonucleotide primers for detecting antibiotic resistance genes in G+ bacteria were also designed from published DNA sequences (GenBank). All primers were developed using the NCBI Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome), Primer3 (http://frodo.wi.mit.edu/primer3/) and ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) [32] software. Primer specificity was verified by sequence alignments using BLAST and NCBI entries. The primers are listed in Tables 3 and 4.

PCR Assays

PCR assays specific for transfer and resistance genes were performed as follows. Each 50-μL PCR reaction mixture contained 1.5 U Taq polymerase (Rapidozym, Berlin, Germany; Agilent Technologies, Böblingen, Germany), 1× PCR buffer, 0.4 μM of each primer, 0.2 mM deoxynucleoside triphosphates, 2–4 mM $MgCl_2$ and template DNA (lysate, genomic or plasmid DNA). DNA amplifications were carried out in a peqSTAR thermocycler (Peqlab, Erlangen, Germany). The temperature profile for resistance genes consisted of an initial denaturation step at 95 °C for 2 min followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 56–58 °C for 30 s and extension at 72 °C for 30 s, followed by an additional 5-min elongation step at 72 °C. The temperature profile for the transfer genes consisted of an initial denaturation step at 95 °C for 2 min followed by 30 cycles of 95 °C for 1 min, 52–56 °C for 1 min, and 72 °C for 1 min and a final elongation at 72 °C for 5 min.

Mating Experiments

The strains for the conjugation assays were selected on basis of their transfer/relaxase genes and antibiotic resistance profile. Strains with relaxase gene, the three signature genes, *virB1*-, *virB4*- and *virD4*-orthologs, and antibiotic resistance genes were selected for biparental matings. Strains encoding only relaxase and resistance genes were selected for triparental mating experiments.

Filter mating experiments were conducted with overnight cultures of donor and recipient that were washed twice in fresh BHI broth, diluted 1:5 in BHI broth and grown separately until OD_{600}=0.5 was reached. For biparental matings, donor and recipient cells were mixed in a 1:10 ratio and for triparental matings, in a 1:1:10 ratio. The mixture was placed on a 0.2-μm sterile nitrocellulose filter cell-side up (Millipore, Schwalbach, Germany) on BHI agar and incubated overnight at 37 °C. Filters were washed with 1 ml 0.9 % sodium chloride and rigorously shaken to detach bacteria from the filters. Serial

Table 3 Oligonucleotides used for the detection of relaxase and transfer genes

Primer	Sequence (5′→3′)	Acc. no.	Position	Reference/source
pIP501 *orf1 fw*	TCGAAGTGGTGAAAAATTGTATAG	L39769	1471-1494	This work
pIP501 *orf1 rev*	AGCATAATGTGAGCATGAGG		1790-1809	
pSK41 *pre fw*	CTGGACTAAAAGGCATGCAA	AF051917	20674-20693	This work
pSK41 *pre rev*	GCAGTTTTCCATCACGCATA		20298-20317	
pSK41 *nes fw*	AGCGCTAGTAGGATTAAAG	AF051917	10016-10034	This work
pSK41 *nes rev*	CATAATAAATGTGCGTGAGG		9706-9725	
pT181 *pre fw*	TCGAACAGAATTATACAGGCAA	CP000045	2708-2729	This work
pT181 *pre rev*	CTGACTTATTTGCTCATGTTTAGC		3082-3105	
pRI1 *mob fw*	TAATCGCTCTAAACGCTGGC	EU370688	3112-3131	This work
pRI1 *mob rev*	ATGAGCGAGAAAAGGCTCTG		2728-2747	
*orf5*_pIP501 *fw*	GTTTTATTTGATCCCGAAGATGAAC	L39769	5641-5664	This work
*orf5*_pIP501 *rev*	GCGGATTGTTGGTTATTTCTTCAA		6274-6295	
*orf7*_pIP501 *fw*	CAATGGGCTACAATTACCGC	AJ505823	605-624	This work
*orf7*_pIP501 *rev*	ACAAGAAGCGTACCGTCTGC		1039-1058	
*orf10*_pIP501 *fw*	TGATTCGATCGGTTGTTCAGGTAG	AJ505823	3660-3683	This work
*orf10*_pIP501 *rev*	CCATTAGATCGAACTTGGGACGGCT		2907-2931	
*traE*_pSK41 *fw*	TATCATTGATCC(T/C)GAA(A/G)ATGAAT	AF051917	27456-27478	This work
*traE*_pSK41 *rev*	TCTTTTGT(T/G)ATTTCGTCCCATAA		28060-28082	
*traG*_pSK41 *fw*	GTGTTGACGGTTCGGGTATC	AF051917	30132-30151	This work
*traG*_pSK41 *rev*	TTTTCCGTCTGAACCTCCAC		30570-30589	
*traK*_pSK41 *fw*	TATCTAAAGACCACCCAGCTAGAG	AF051917	34636-34660	This work
*traK*_pSK41 *rev*	TACTTGTTTCAAACTCTACAGTAGC		35185-35209	
*traL*_pSK41 *fw*	ATGGGGACTATGGCAGGTAG	AF051917	36279-36298	This work
*traL*_pSK41*rev*	AAGTTTTGCACCACTTCCAG		36667-36686	
*traM*_pSK41*fw*	TGTTGTATGGGGAAAACAAGC	AF051917	36870-36890	This work
*traM*_pSK41*rev*	GCTGGGCTTATAGC(A/G)TCATC		37051-37070	

dilutions of the cells were plated on BHI agar with appropriate antibiotics to enumerate recipients and transconjugants, respectively. Mobilisation and transfer rates were calculated as transconjugants per recipient; values are the mean of three independent measurements (unless otherwise indicated) with standard deviation.

Biofilm Screening

To test the isolates for biofilm formation, a quantitative adherence assay [33], with modifications by Klingenberg et al. [34], was used. This method is widely used to evaluate biofilm formation by a variety of organisms [35–38]. Briefly, 200 µl of BHI, THB (Todd Hewitt broth) and tryptic soy broth (Becton Dickinson, Heidelberg, Germany), medium in 96-well flat-bottom MT polystyrene plates were inoculated with 10 µl overnight cultures of the bacteria to be tested and grown without shaking at room temperature for 24 h. Adherent bacteria were fixed by air drying at 55 °C for 1 h and then stained with 0.4 % crystal violet (Merck, Darmstadt, Germany). *Bacillus cereus s.l.* A1 [39], *S. aureus* G7 and *S. aureus* H2 were used as positive controls due to abundant biofilm formation in the biofilm reactors (data not shown). As negative control, the respective media were used. The optical density of the biofilms was measured in a microplate reader at 570 nm in triplicate for each strain. The ability to form biofilm was scored as follows—OD<0.120, no biofilm formation, 0.120<OD<0.240, weak biofilm formation, OD>0.240, strong biofilm formation [33, 36].

Results and Discussion

The Antarctic Research Station Concordia and the ISS are confined habitats in extreme and hostile environments, both isolated from human civilisation and with low crew turnover. Microgravity and radiation from outer space are the most important differences between ISS and Concordia. Nonetheless, confined habitats like Concordia are used as a model environment for long-duration spaceflights to study human adaptation to isolated and confined extreme environmental situations as they allow to map and monitor the dynamics of airborne bacteria over a certain period.

In this study, *Staphylococcus* and *Enterococcus* species isolated from the ISS (29 isolates) and Concordia (55 isolates) were investigated regarding their antibiotic resistance pattern phenotypically and on a molecular scale. Furthermore, their extrachromosomal DNA content was analysed as well as the potential transfer of antibiotic resistance genes via HGT.

Table 4 Oligonucleotides used for the detection of antibiotic resistance genes

Primer	Sequence (5′→3′)	Acc. no.	Position	Reference/source
aac6-aph2a fw	GCCAGAACATGAATTACACGAG	NC_005024	42981-43002	This work
aac6-aph2a rev	CTGTTGTTGCATTTAGTCTTTCC		43569-43591	
*aadD*_pSK41 *fw*	TGTCGTTCTGTCCACTCCTG	AF051917	21901-21920	This work
*aadD*_pSK41 *rev*	ATGAATGGACAACCGGTGAG		22407-22426	
aph(2)-Ib fw	AGGATGCCCTTGCATATGATGAAGCGACGT	AF207840	438-467	[81]
aph(2)-Ib rev	ATCAGCATAAGGCGCCGGAAGTAGCAGAAA		858-887	
aph(2)-Ic fw	AGCATACAATCCGTCGAGTCGCTTGGTGAG	U51479	253-282	[81]
aph(2)-Ic rev	CTGGCGCTGCAACTTGCTGAGTTCATGAAT		541-570	
aph(2)-Id fw	GCCATCAGAAACGTACCAAATGTCTTTCGCAGG	AF016483	379-411	[81]
aph(2)-Id rev	GGCAGCTAAGGACCTGGCCCGATTTCTAAG		484-513	
ampC fw	GTGACCAGATACTGGCCACA	AJ005633	368-387	[82]
ampC rev	TTACTGTAGCGCCTCGAGGA		1170-1189	
aph(3′)-III fw	CCGCTGCGTAAAAGATAC	X92945	39555-39572	This work
aph(3′)-III rev	GTCATACCACTTGTCCGC		40147-40164	
cat_{pC194} *fw*	CGACGGAGAGTTAGGTTATTGG	NC_002013	1478-1499	This work
cat_{pC194} *rev*	GGCCTATCTGACAATTCCTG		1872-1891	
cat_{pIP501} *fw*	TGGGATAGAAAAGAATATTTTGAACAC	X65462	238-264	This work
cat_{pIP501} *rev*	TCCAAGGAATCATTGAAATCG		626-646	
cat_{LM} *fw*	CTAAAATCAATCCAAGGAATCATCG	X92946	22115-22139	This work
cat_{LM} *rev*	GGATATGAACTGTATCCTGCTTTG		22405-22428	
*erm*B *fw*	GCATTTAACGACGAAACTGGCT	U00453	6796-6817	[82]
*erm*B *rev*	GACAATACTTGCTCATAAGTAATGGT		7343-7368	
*erm*C *fw*	CGTAACTGCCATTGAAATAGACC	V01278	2555-2577	This work
*erm*C *rev*	TCCTGCATGTTTTAAGGAATTG		2079-2100	
*erm*D *fw*	CGGGCAAATATTAGCATAGACG	M29832	544-565	This work
*erm*D *rev*	ATTCTGACCATTGCCGAGTC		988-1007	
*erm*G *fw*	TGCAGGGAAAGGTCATTTTAC	M15332	785-805	This work
*erm*G *rev*	AACCCATTTCATTACAAAAGTTTC		1245-1268	
*mec*A *fw*	TAATAGTTGTAGTTGTCGGGTTTG	X52593	174-195	[82]
*mec*A *rev*	TAACCTAATAGATGTGAAGTCGCT		881-904	
*tet*K_pT181 *fw*	TTTGAGCTGTCTTGGTTCATTG	CP000045	1398-1419	This work
*tet*K_pT181 *rev*	AGCCCACCAGAAAACAAACC		1918-1937	
*tet*M *fw*	GAACTCGAACAAGAGGAAAGC	M85225	1114-1134	[83]
*tet*M *rev*	ATGGAAGCCCAGAAAGGAT		1835-1853	
*tet*O *fw*	GGATGGCATACAGGCACAGA	M18896	512-531	[82]
*tet*O *rev*	GTTTGGATCATAGGGAGAGGAT		1228-1249	
*tet*S_pK214 *fw*	TGGTCAACGGCTTGTCTATG	X92946	27158-27177	This work
*tet*S_pK214 *rev*	AGCCCAGAAAGGATTTGGAG		26631-26650	
*van*A *fw*	GAAATCAACCATGTTGATGTAGCA	AF516335	4006-4029	[82]
*van*A *rev*	TTTGCCGTTTCCTGTATCCGT		4557-4577	
*van*B *fw*	CCCGAATTTCAAATGATTGAAAA	L06138	113-135	[84]
*van*B *rev*	CGCCATCCTCCTGCAAAA		552-569	
*van*C1/2 *fw*	ATGGATTGGTA(C/T)T(G/T)GTAT	AF162694/L29638	133-150/142-159	[85]
*van*C1/2 *rev*	TAGCGGGAGTG(A/C)C(C/T)(A/C)GTAA		929-947/950-968	

Most members of the bacterial community of the ISS and Concordia belonged to the class of Bacilli with 86 % and 52 % (as percentage of total number of isolates), respectively. Staphylococci (87.6 % and 55.8 % respectively) represented the most dominant member of the Bacilli (Table 2). The staphylococci isolates mainly belong to the coagulase-negative staphylococci (CNS), namely 90.3 % of the Concordia and 71 % of the ISS staphylococci. Among the CNS, several opportunistic pathogens were isolated, including *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* and *Staphylococcus saprophyticus*. *S. aureus* represented 3.4 % of the Concordia and 1.8 % of the ISS staphylococci. Only 12 *E. faecalis* strains isolated from the ISS were characterised in this work due to low abundance of *Enterococcus* species in the sampling campaigns. Altogether 13 (4.3 % of the isolates) *E. faecalis* strains were

isolated from the ISS (12 from air samples and one from a crew member) in contrast to only one (0.4 % of the isolates) *Enterococcus casseliflavus* strain from Concordia (Table 2) [1, 2].

Although the bacterial community of the ISS and Concordia appear to be similar, when interpreting different studies, it is crucial to recall the experimental results relative to the different methods. Differences in the analysis protocol such as sampling method [40, 41], storage [18] and diversity assessment [40] can affect the outcome regarding population size and diversity.

Among the isolated genera from ISS, *Staphylococcus* was the most abundant. Results from the ISS and MIR space station suggest that bacteria of human origin are the most commonly recovered bacterial genera from air and surface samples. Novikova [42] isolated from MIR space station in addition to *Staphylococcus* species *Bacillus* and *Corynebacterium* species. However, Enterococci were isolated only occasionally [42]. Results from non-confined environments support these results; G+ cocci were the most abundant bacteria isolated from airborne samples taken at different places like restaurants and underground stations, most probably originating from humans [5, 6, 43, 44].

Most of the staphylococcal and enterococcal species described in this study are opportunistic pathogens. *S. aureus* is an important cause of nosocomial wound and catheter-related infections, pneumonia and bacteremia/sepsis as well as community-acquired (CA) skin and soft tissue infections [45, 46]. *S. epidermidis*, *S. haemolyticus* as well as *E. faecalis* are important nosocomial pathogens causing for instance bacteremia and urinary tract infections [47, 48]. *S. saprophyticus* is an important cause of ambulant urinary tract infections [49], and *S. lugdunensis* occasionally causes serious infections including prosthetic joint infections, endocarditis and septicemia [50].

Stressors of space flight environment as well as Concordia-like confined environments, e.g. microgravity, radiation, isolation, microbial contamination and sleep disruption, have been shown to cause immune suppression and inflammatory responses. Therefore, opportunistic pathogens like staphylococci and enterococci are of potential threat to crew members working under these confined and stressful conditions, with the risk of infectious disease occurrence increasing with longer mission duration [10, 51–53]. Treatability of infectious diseases, especially in confined habitats with no access to intensive care units and only limited amounts of antibiotics available, is therefore a major concern.

Resistances to three, up to five antibiotics were detected in several staphylococcal and enterococcal strains from ISS and Concordia investigated in this study. Resistance was detected in 86.2 % (25) of the ISS and in 43.6 % (24) of the Concordia isolates. Most antibiotic resistances were detected in *E. faecalis* strains isolated from the ISS (Table 5). All ISS *E. faecalis* isolates were chloramphenicol-, erythromycin-, high-level streptomycin-, kanamycin- and tetracycline-resistant.

The resistant isolates were screened for the presence of 22 different antibiotic resistance genes involved in resistance to aminoglycosides (*aac(6')-Ie-aph(2')-Ia*, *aph(2')-Ib*, *aph(2')-Ic*, *aph(2')-Id*, *aph(3')-III*, *ant(4')-Ia*), beta-lactams (*mec*A, *amp*C), glycopeptides (*van*A, *van*B, *van*C1/2), macrolide-lincosamide-streptogramins (*erm*B, *erm*C, *erm*D, *erm*G), tetracycline (*tet*K, *tet*M, *tet*O, *tet*S) and chloramphenicol (cat_{pC194}, cat_{pIP501}, cat_{LM}).

Most resistance genes were detected in the ISS isolates. In 68 % (17) of the resistant strains, two to five resistance genes were detected. Four *E. faecalis* strains encode five, and two *E. faecalis* strains and one *S. hominis* subsp. *hominis* strain (F3) four resistance genes. On the contrary, in only 33.3 % (8) of the resistant Concordia isolates, two or three antibiotic resistance genes were detected (Table 5). Only one Concordia strain, *S. epidermidis* G16, encodes four resistance genes. In total, 14 out of 22 selected antibiotic resistance genes were identified in the ISS and Concordia isolates. *van*A, *van*B and *van*C1/2 were not detected in any of these strains.

The most prevalent resistance genes among the staphylococci from ISS and Concordia were *erm*C, *tet*K, cat_{pIP501}, cat_{LM} and cat_{pC194} encoding macrolide, tetracycline and chloramphenicol resistances, respectively. These genes are plasmid-borne, confer resistance to inhibitors of bacterial protein biosynthesis and have been identified in staphylococci of human and animal origin [54].

At least six plasmid-encoded *erm* genes of the classes A, B, C, T, Y and 33 are known in staphylococci [54]. In agreement with a previously reported low *ermB* prevalence in *Staphylococcus* [55], the *erm*B resistance gene was not detected in any ISS and Concordia *Staphylococcus* isolate.

However, *erm*D and *erm*G, originally detected on the chromosome of *Bacillus licheniformis* and *Bacillus sphaericus*, respectively [56, 57], were mostly found in the Concordia staphylococci isolates. To date, *erm*D has only been found in *Bacillus* species [58] whereas the *erm*G gene was previously detected in *Staphylococcus*, *Bacteroides* and *Clostridium* species [59, 60].

To analyse the ISS and Concordia isolates for mobile genetic elements, we developed PCR-based screening assays for relaxases of the MOB_Q (pIP501, pRE25, pSK41, pGO1) and MOB_V family (pT181, pRI1, pSK41, pGO1) of plasmids from G+ bacteria [29–31]. These relaxase families were selected due to their prevalence on conjugative plasmids from staphylococci and enterococci/streptococci capable of transferring resistance genes and mobilising plasmids into different genera [61]. Moreover, mobilisable plasmids from the Mob_V family were selected since they can be mobilised by conjugative plasmids from G+ and G− bacteria as well as by conjugative transposons [29].

PCR assays were also designed to detect potential conjugative plasmids via primer pairs specific for *virB1*-,

Table 5 Antibiotic resistance profiles and genes of the ISS and Concordia isolates

Strain no.	Species	Sample name	Antibiotic resistance phenotype	Genotype[a]	Sampling year
	ISS air samples				
E3	*S. aureus* (16S)	R42	E, K	*ant(4')-Ia*	2002
E4	*S. hominis* subsp. *hominis* (16S)	R44	E, T	*erm*C, *tet*K	2002
E5	*S. epidermidis*	R56	C	cat_{pIP501}	2002
E6	*S. epidermidis* (16S)	R61	C	cat_{pIP501}, *aac(6')-Ie-aph(2')-Ia*,	2002
E7	*S. haemolyticus* (16S)	R69	E, T	*erm*C, *tet*K	2002
E9	*S. lugdunensis* (16S)	R76	C	cat_{pC194}	2002
E11	*S. epidermidis*	Plate5iso2 (p5i2)	C	cat_{LM}	2003
E12	*S. epidermidis*	Plate 8 iso1	C, T	*tet*K	2003
E13	*E. faecalis*	Plate 10 iso2	C, E, S, K, T	*aph(3')-III*, *erm*B, *tet*M, cat_{pIP501}	2003
E14	*E. faecalis*	Plate 10 iso3	C, E, S, K, T	*aph(3')-III*, *erm*B, *erm*D, *tet*M, cat_{pIP501}	2003
E15	*E. faecalis*	Plate 10 iso4	C, E, S, K, T	*aph(3')-III*, *tet*M, cat_{pIP501}	2003
E16	*E. faecalis*	Plate 10 iso5	C, E, S, K, T	*aph(3')-III*, *erm*B, *erm*D, *tet*M, cat_{pIP501}	2003
E17	*E. faecalis*	Plate 10 iso7	C, E, S, K, T	*aph(3')-III*, *erm*B, *erm*D, *tet*M, cat_{pIP501}	2003
E18	*E. faecalis*	Plate 10 iso8	C, E, S, K, T	*aph(3')-III*, *erm*B, *tet*M, *tet*O, cat_{pIP501}	2003
E19	*E. faecalis*	Plate 10 iso9	C, E, S, K, T	*aph(3')-III*, *erm*B, *tet*M, cat_{pIP501}	2003
E20	*E. faecalis*	Plate 10 iso10	C, E, S, K, T	*aph(3')-III*, cat_{pIP501}	2003
E21	*E. faecalis*	Plate 10 iso11	C, E, S, K, T	*aph(3')-III*, *tet*M, cat_{pIP501}	2003
E22	*S. epidermidis*	Plate 10 iso12	C, K	*aph(3')-III*	2003
E23	*E. faecalis*	Plate 10 iso14	C, E, S, K, T	*aph(3')-III*, *tet*M, cat_{pIP501}	2003
E24	*E. faecalis*	Plate 10 iso16	C, E, S, K, T	*aph(3')-III*, *tet*M, cat_{pIP501}	2003
E25	*E. faecalis*	Plate 10 iso18	C, E, S, K, T	*aph(3')-III*, *tet*M, cat_{pIP501}	2003
	ISS crew samples				
F1	*S. lugdunensis* (16S)	4IJ-4	C	cat_{pC194}	2006
F2	*S. haemolyticus* (16S)	5IL-2	E, T	*erm*C, *tet*K	2006
F3	*S. aureus* (16S)	6OD-2	A, E	*erm*C	2006
F4	*S. hominis* subsp. *hominis* (16S)	7IL-2	C, E, T	*erm*C, *erm*G, *tet*K, cat_{pC194}	2006
	Concordia air samples				
G1	*S. hominis* subsp. *hominis*	T0-Air1-1	C	n.d.	2004
G2	*S. hominis* subsp. *hominis*	T2-Air5-3	E, T	*erm*C, *tet*K	2005
G4	*S. hominis* subsp. *hominis*	T3-Air3-4	A, E, T	*erm*C, *tet*K	2005
G5	*S. warneri*	T3-Air4-1	A, E	n.d.	2005
G6	*S. cohnii subsp. urealyticum*	T4-Air6-3	E, T	n.d.	2005
G7	*S. aureus*	T5-Air3-1	A	n.d.	2005
G8	*S. epidermidis*	T5-Air4-3	K, T	n.d.	2005
G10	*S. epidermidis*	T6-Air1-4	C	n.d.	2005
G13	*S. haemolyticus*	T8-Air7-1	G	n.d.	2005
G14	*S. hominis* subsp. *hominis* (16S)	T9-Air2-4	C, E	n.d.	2005
G15	*S. epidermidis*	T10-Air8-2	T	n.d.	2005
G16	*S. epidermidis*	T11-Air2-8	C, E, T	*erm*C, *tet*M, *tet*K, cat_{LM}	2005
G17	*S. saprophyticus*	T11-Air3-3	A, E	n.d.	2005
G18	*S. epidermidis* (16S)	T11-Air4-3	C, E	*erm*D, cat_{pC194}, *aac(6')-Ie-aph(2')-Ia*	2005
G19	*S. capitis* subsp. *capitis* (16S)	T11-Air4-7	C, A, E	*erm*C, *mec*A, cat_{LM}	2005
G20	*S. hominis* subsp. *hominis* (16S)	T12-Air2-1	C, E, T	*erm*D, cat_{pC194}, cat_{LM}	2005
G21	*S. hominis* subsp. *hominis* (16S)	T12-Air2-2	C	n.d.	2005
	Concordia crew samples				
H3	*Staphylococcus.* sp.	T0-C4-HA28-3	C	cat_{pC194}	2005
H4	*S. epidermidis* (16S)	T0-C6-HA28-3	E	*erm*C, *erm*D	2005
H5	*S. pasteuri*	T0-C6-HA28-5	A, E	*erm*C	2005
H6	*S. hominis* subsp. *hominis* (16S)	T0-C1-HA37-1	S	n.d.	2005
H8	*S. hominis* subsp. *hominis* (16S)	T0B-C4-HA28-4	E, T	n.d.	2004
H9	*S. haemolyticus* (16S)	T0B-C2-HA37-3	A, E	*aph(3')-III*, *erm*C	2004
H11	*S. aureus*	T0B-C4-HA37-3	A	n.d.	2004

A ampicillin, *C* chloramphenicol, *E* erythromycin, *G* gentamycin, *K* kanamycin; *S* streptomycin, *T* tetracycline, *(16S)* species was identified by 16S rDNA sequencing, *n.d.* not detected

[a] *aac(6')-Ie-aph(2')-Ia*: gentamycin; *ant(4')-Ia*, *aph(3')-III*: kanamycin; *cat*: chloramphenicol; *erm*: erythromycin; *mec*: methicillin; *tet*: tetracycline resistance genes

virB4- and *virD4*-like genes from conjugative plasmids (pIP501, pRE25, pSK41, pGO1, pSM19035, pMRC01) from G+ bacteria. The relaxase and transfer gene profiles of selected (at least one relaxase and one resistance gene are present) ISS and Concordia strains are listed in Table 6.

Relaxase genes belonging to the MOB_V and MOB_Q family were detected in 75.9 % (22) of the ISS and in 50.9 % (28) of

Table 6 Resistance genes, transfer genes, plasmids and biofilm formation of selected strains from ISS and Concordia

Strain no.	Species	Sample name	Genotype[a]	Plasmids[b]		Biofilm formation
				>20 kb	<20 kb	
	ISS air samples					
E1	*S. epidermidis* (16S)	R12	pre_{pT181}, nes_{pSK41},pre_{pSK41}, *traE*, *traG, traK*, *traM*, *traL*	4	8	++
E2	*S. saprophyticus* (16S)	R13	nes_{pSK41}, *traE*, *traG*, *traK*, *traM*, *traL*	4	4	++
E4	*S. hominis* subsp. *hominis* (16S)	R44	pre_{pT181}, *erm*C, *tet*K	n. d.	4	−
E7	*S. haemolyticus* (16S)	R69	pre_{pT181}, *traE*, *erm*C, *tet*K	n. d.	7	+
E8	*S. epidermidis*	R75	pre_{pSK41}, pre_{pT181}, *tet*K	n. d.	n. d.	++
E9	*S. lugdunensis*	R76	*traE, traG, traK, traM*, *traL*, cat_{pC194}	n. d.	2	++
E11	*S. epidermidis*	Plate5iso2	pre_{pSK41}, pre_{pT181}, cat_{LM}	n. d.	4	+
E12	*S. epidermidis*	Plate 8 iso1	pre_{pSK41}, pre_{pT181}, *tet*K	3	0	+
E13	*E. faecalis*	Plate 10 iso2	pre_{pSK41}, *aph(3′)-III*, *erm*B, *tet*M, cat_{pIP501}	1	0	+
E14	*E. faecalis*	Plate 10 iso3	pre_{pSK41}, *aph(3′)-III*, *erm*B, *erm*D, *tet*M, cat_{pIP501}	1	0	++
E15	*E. faecalis*	Plate 10 iso4	pre_{pSK41}, *aph(3′)-III*, *tet*M, cat_{pIP501}	1	0	++
E16	*E. faecalis*	Plate 10 iso5	pre_{pSK41}, *orf7*, *orf10*, *aph(3′)-III*, *erm*B, *erm*D, *tet*M, cat_{pIP501}	1	0	++
E17	*E. faecalis*	Plate 10 iso7	pre_{pSK41}, *aph(3′)-III*, *erm*B, *erm*D, *tet*M, cat_{pIP501}	1	0	+
E18	*E. faecalis*	Plate 10 iso8	pre_{pSK41}, *aph(3′)-III*, *erm*B, *tet*M, *tet*O, cat_{pIP501}	1	0	−
E19	*E. faecalis*	Plate 10 iso9	pre_{pSK41}, *orf7*, *aph(3′)-III*, *erm*B, *tet*M, cat_{pIP501}	1	0	−
E20	*E. faecalis*	Plate 10 iso10	pre_{pSK41}, *aph(3′)-III*, cat_{pIP501}	1	0	−
E21	*E. faecalis*	Plate 10 iso11	pre_{pSK41}, *aph(3′)-III*, *tet*M, cat_{pIP501}	1	0	−
E22	*S. epidermidis*	Plate 10 iso12	pre_{pSK41}, *aph(3′)-III*	4	8	+
E23	*E. faecalis*	Plate 10 iso14	pre_{pSK41}, *aph(3′)-III*, *tet*M, cat_{pIP501}	1	0	+
E24	*E. faecalis*	Plate 10 iso16	pre_{pSK41}, *aph(3′)-III*, *tet*M, cat_{pIP501}	1	0	+
E25	*E. faecalis*	Plate 10 iso18	pre_{pSK41}, *aph(3′)-III*, *tet*M, cat_{pIP501}	1	0	++
	ISS crew samples					
F1	*S. lugdunensis*	4IJ-4	*traE, traG, traK, traM,* cat_{pC194}	n. d.	n. d.	+
F2	*S. haemolyticus* (16S)	5IL-2	*traE, traG, traK, traM*, *traL, erm*C, *tet*K	1	2	+
F4	*S. hominis* subsp. *hominis* (16S)	7IL-2	nes_{pSK41}, pre_{pT181}, *traE, traG, traK, traM*, *traL, erm*C, *erm*G, *tet*K, cat_{pC194}	n. d.	5	+
	Concordia air samples					
G2	*S. hominis* subsp. *hominis*	T2-Air5-3	pre_{pT181}, nes_{pSK41}, pre_{pSK41}, *erm*C, *tet*K	2	8	+
G4	*S. hominis* subsp. *hominis*	T3-Air3-4	pre_{pT181}, *erm*C, *tet*K	1	2	+
G12	*S. hominis* subsp. *hominis* (16S)	T6-Air2-3	nes_{pSK41}, cat_{pIP501}	n. d.	8	+
G16	*S. epidermidis*	T11-Air2-8	pre_{pT181}, pre_{pSK41}, *traK*, *erm*C, *tet*M, *tet*K, cat_{LM}	2	8	++
G18	*S. epidermidis* (16S)	T11-Air4-3	nes_{pSK41}, *erm*D, cat_{pC194}, *aac6-aph2a*	3	1	+
G19	*S. capitis* subsp. *capitis* (16S)	T11-Air4-7	pre_{pSK41}, *erm*C, *mec*A, cat_{LM}	1	6	−
	Concordia crew samples					
H3	*Staphylococcus* sp. (16S)	T0-C4-HA28-3	pre_{pSK41}, *traE*, cat_{pC194}	1	3	++
H4	*S. epidermidis* (16S)	T0-C6-HA28-3	pre_{pSK41}, *erm*C, *erm*D	1	4	++

(16S) species was identified by 16S rDNA sequencing, *n.d.* not determined, − no biofilm, + weak biofilm formation, ++ strong biofilm formation

[a] *aac6-aph2a*: gentamycin; *aph(3′)-III*: kanamycin; cat_{pIP501}/cat_{pC194}/cat_{LM}: chloramphenicol; *erm*B/*erm*C/*erm*D/*erm*G: erythromycin, *mec*A: methicillin; *tet*K/*tet*M/*tet*O: tetracycline resistance genes

[b] The numbers correspond to the numbers of bands observed on 0.7 % or 1.0 % agarose gel

the Concordia strains investigated in this study. Four of the ISS strains and two of the Concordia strains encode two or three relaxase genes. In 31 % (9) of the ISS strains, belonging to CNS and *Enterococcus* species and in 7.3 % (4) of the Concordia strains (CNS) pSK41- and pIP501-like transfer genes were detected. In six ISS strains (CNS: E1, E2, E9, F1, F2, F3), three pSK41-like transfer genes, *traE*, *traG* and *traK*, were detected. Gene transfer experiments verified that one of these strains (*S. haemolyticus* F2) contains a conjugative element. The antibiotic resistance gene *erm*C was successfully transferred to *E. faecalis* OG1X.

The staphylococcal *erm*C gene can be encoded both on non-mobilisable plasmids, e.g. pKH19 (2.4 kb), pKH20 (2.4 kb) and pPV141 (2.4 kb), and on plasmids encoding *mob*-genes (Mob_V class), like pE194 (3.7 kb) and pSES22 (4 kb). Furthermore, the *ermC* gene can be clustered with the mupirocin resistance gene *ileS2* on the conjugative pSK41-like plasmid pUSA03, isolated from CA MRSA USA300 [31, 62]. Here, the *ermC* gene was exclusively found in combination with at least one *mob*-gene and in two cases with additional pSK41-like type IV secretion system (T4SS) genes (isolates F2, *S. haemolyticus* and F4, *S. hominis* subsp. *hominis*). Interestingly, the *ermC* gene of isolate F2 (ISS crew sample) was biparentally transferred to *E. faecalis*, indicating a potential conjugative element in F2 that could be related to pSK41 or pUSA03. Speculatively, this transfer could also have happened in multi-species biofilms within the confined ISS/Concordia habitats, thus transferring the *ermC* gene with other traits into more pathogenic bacteria like *S. aureus*. Our results though suggest that, at least among the *S. aureus* isolates we investigated, such a transfer did not occur.

pSK41-like *vir* signature genes were also detected in ISS staphylococcal isolates together with chloramphenicol resistance genes (E9, F1). Due to the absence of plasmids larger than 20,000 bp, isolates E9 and F1 were not applied to mating experiments; thus, it remains speculative if these two isolates possess conjugative/mobilisable elements that enable transfer of the chloramphenicol resistance genes.

Among the Concordia isolates, six (four airborne and two crew samples) may contain mobilisable *erm*C-, *tet*K- and *cat*-carrying plasmids. For the isolate G2 (*S. hominis* subsp. *hominis*; pre_{pT181}, nes_{pSK41}, pre_{pSK41}, *erm*C, *tet*K), mobilisation of the *tet*K and pre_{pT181} genes was experimentally proved from *S. hominis* subsp. *hominis* to *S. aureus*. The tetracycline resistance gene *tet*K is commonly encoded on structurally closely related plasmids of about 4.5 kb, like pT181 (Mob_V class) from *S. aureus*. pT181-like plasmids are often integrated into larger plasmids via IS*257* elements [54]. Thus, *tet*K and pre_{pT181} genes in isolate G2 could be derived from a pT181-like plasmid.

The most prevalent resistance genes detected in the ISS *E. faecalis* strains were *erm*B, *tet*M, *aph(3')-III* and cat_{pIP501}. They are usually located on transposons of the Tn*916*-Tn*1545* family [14, 63]. *erm*B is also encoded on conjugative plasmids like pIP501 (30.6 kb; cat_{pIP501}), pRE25 (50.2 kb; cat_{pIP501}, *aph(3')-III, sat4*, *ant(6)-Ia*), pAMβ1 (27.8 kb) and the pheromone-responsive plasmids pTEF1 (66.3 kb; *aac6-aph2a*) and pTW9 (85 kb; *van*A) [17, 19, 64]. Due to their limited host range, these pheromone-responsive plasmids were not focus of our study.

Zhu et al. [65] recently discovered that the emergence of vancomycin-resistant Enterococci is related to Inc18-like plasmids that show high (up to nearly 100 %) similarity to the pIP501 *tra* region. Among all the ISS *E. faecalis* isolates, plasmids in the size range of 40 to 130 kb were

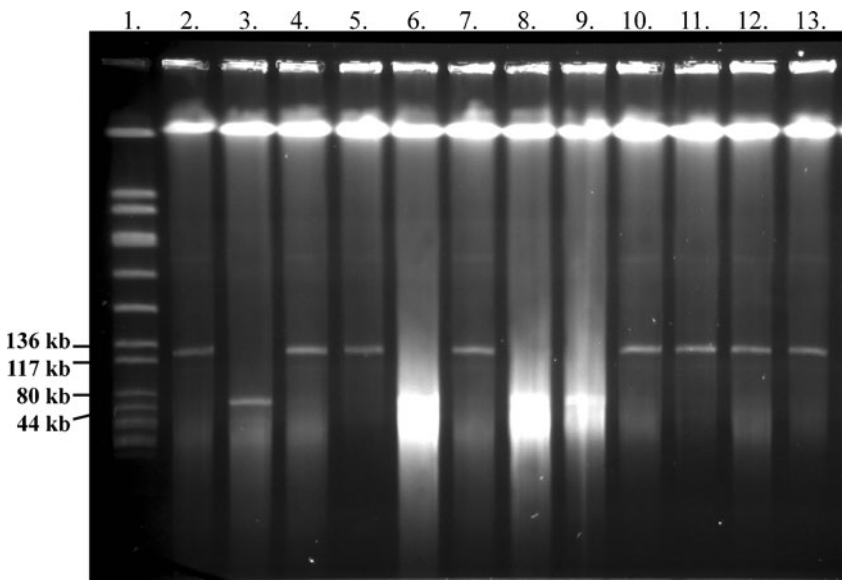

Fig. 1 *Lane 1*: *Sma*I-digested DNA of *S. aureus* NCTC 8325 (673, 361, 324, 262/257, 208, 175, 136, 117, 80, 44, 36, and 10 kb); *lane 2*: isolate E20; *lane 3*: isolate E21; *lane 4*: isolate E23; *lane 5*: isolate E24; *lane 6*: isolate E25; *lane 7*: isolate E13; *lane 8*: isolate E14; *lane 9*: isolate E15; *lane 10*: isolate E16; *lane 11*: isolate E17; *lane 12*: isolate E18; *lane 13*: isolate E19

detected (Fig. 1), but only for one isolate (E16) T4SS traits were found with similarity to the pIP501 *tra* region (*virB1*-like $orf7_{pIP501}$, *virD4*-like $orf10_{pIP501}$) as well as the pSK41 *pre* relaxase gene and the resistance determinants, *aph(3')-III*, *erm*B, *erm*D, *tet*M and cat_{pIP501}, indicating a likely capability to transfer DNA.

The question whether HGT can occur under space flight conditions and microgravity has been studied recently. De Boever et al. [66] conducted gene transfer experiments aboard the ISS during the Soyuz Mission 8S. Whereas for G− bacteria no differences in HGT frequencies were detectable between ground and space flight experiments, the study suggested a possible effect on plasmid transfer in *Bacillus* species. On the other hand, Beuls et al. [67] used microgravity simulators for gene transfer assays between two *Bacillus thuringiensis* strains. There was no significant difference detectable between HGT frequencies with or without microgravity. These data suggest that HGT occurs under microgravity with putative species-dependent differences. Nonetheless, the fact that 83 % of the ISS and 92.7 % of the Concordia strains investigated in this study form biofilm under standard laboratory conditions and microgravity probably enhancing biofilm production in outer space during space flights [e.g. 9, 68, 69] emphasises the need to analyse the mobilome [12] in these (outer space) confined habitats. A related survey focusing on *Bacillus* species was recently published analysing ISS and Concordia isolates regarding virulence and transfer capabilities [39]. In summary, knowledge on the prevalence of MGEs and resistance genes in the bacterial population aboard is required to evaluate the potential threat for the human crew posed by the microbial community in these confined habitats.

Conclusions

In synopsis with the data from Van Houdt et al. [2] on the airborne population of Antarctic base Concordia, we conclude that the microbial community in the studied confined habitats is to a large extent comparable with the microorganisms detected in unconfined environments, such as apartment buildings and hospitals. Most of the microbes originate from the crew.

The high incidence of resistant *Staphylococcus* and *Enterococcus* isolates from ISS and Concordia can pose an increased health risk due to consequent treatment complications after infection. Therefore, periodical control of the microbial community in confined environments by fast and reliable methods such as species-specific and antibiotic resistance PCR is recommended.

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