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RESEARCH ARTICLE

REVISED Microbiome of a cruise ship: ESKAPE bacteria are commonly found on surfaces and crew members' hands

[version 2; peer review: 1 approved with reservations, 2 not approved]

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v2 **First published:** 05 Jun 2025, **5**:153
https://doi.org/10.12688/openreseurope.19710.1
Latest published: 27 Feb 2026, **5**:153
https://doi.org/10.12688/openreseurope.19710.2

Abstract

Cruise ships are relatively small, crowded spaces where infection control is especially important. There is limited information available about the composition of the microbiome found on frequently-touched surfaces in cruise ships. This study describes the microbial composition of cruise ship surfaces and crew member skin microbiomes. Fingertips of crew members and surfaces were sampled using agar plates. After culturing, DNA was extracted and sequenced using Nanopore long-read whole metagenome sequencing. Classification was performed using Kraken2, and antibiotic resistance genes were identified using the CARD RGI Resistance Gene Identifier. In total, 10'369'710 reads were generated. In the 15 hand samples, 2'495 species were detected. 99.9% of the samples were identified as bacterial, which indicates a limitation of the extraction methods. The majority of the hand samples belonged to the *Staphylococcus* genus (69.38%). Significant individual differences were observed in the microbiome composition of each sample. For the surface samples, 22 species that were present in all or almost all surfaces were listed. Among them, many potentially dangerous pathogenic bacteria were identified, such as *Acinetobacter baumannii*, *Enterobacter* species, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus.* Their antimicrobial resistance genes were also detected in the analyzed sample. Surface samples had a similar composition to hand samples, indicating a strong correlation. In both cases, known pathogens were frequently isolated, suggesting that these bacteria are present throughout our environment. This highlights the importance of regular cleaning and disinfection in crowded areas, such as cruise

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Approval Status ✗ ✗ ?

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version 2 (revision) 27 Feb 2026			? view
version 1 05 Jun 2025	✗ view	✗ view	

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ships.

Key findings

Diverse Microbiomes

2,495 bacterial species were found in the hand samples, with large individual differences.

The most common bacteria on hands were from the Staphylococcus genus, making up 69% of the sample.

Bacterial overlap between hands and surfaces

The bacteria on surfaces closely resembled those found on hands, suggesting frequent transfer between humans and the environment.

22 bacterial species were present on almost all surfaces, including potential pathogens like Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, and Acinetobacter baumannii.

Antibiotic Resistance Genes

Bacteria on the ship contained genes that can make them resistant to antibiotics, raising concerns about their spread in crowded settings.

These resistance genes were found even on regularly cleaned surfaces, indicating that cleaning alone may not fully eliminate resistant bacteria.

Implications

This study highlights how bacteria move between people and the environment, emphasizing the need for improved hand hygiene and thorough disinfection. While the ship's cleaning protocols were in place, potentially harmful bacteria and antibiotic-resistant genes were still detected, showing the challenge of maintaining a microbially safe environment.

Plain language Summary

Microbiome of a Cruise Ship: Bacteria on Surfaces and Crew Members' Hands

Cruise ships are enclosed, high-traffic environments where bacteria can spread easily. This study investigated the microbiome—microorganisms living on surfaces and human skin—on a cruise ship by analyzing bacteria found on frequently touched surfaces and crew members' fingertips.

Researchers collected samples from crew members' hands and various surfaces on the ship, such as stair rails, door handles, and elevator buttons. The bacteria were grown in the lab and analyzed

using DNA sequencing. The goal was to determine which bacteria were present and whether they carried antibiotic resistance genes.

Keywords
hand microbiome, hand hygiene, environmental sampling, surface samples, community settings, frequently-touched surfaces

This article is included in the Horizon Europe gateway.

This article is included in the Bacterial Pathogenesis collection.

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Author roles: Bansaghi S: Conceptualization, Data Curation, Formal Analysis, Investigation, Validation, Visualization, Writing – Original Draft Preparation; **Járay T**: Data Curation, Formal Analysis, Software, Writing – Review & Editing; **Gulyás G**: Data Curation, Formal Analysis, Software; **Tombácz D**: Writing – Review & Editing; **Boldogkői Z**: Writing – Review & Editing; **Klein J**: Conceptualization, Data Curation, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This project has received funding from the European Union's Horizon Europe under grant agreement No 101069764 (project name HEALTHY SAILING: Prevention, mitigation, management of infectious diseases on cruise ships and passenger ferries. DT has received funding from the Lendület (Momentum) Programme of the Hungarian Academy of Sciences under grant agreement No LP2020-8/2020.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Bansaghi S, Járay T, Gulyás G *et al.* **Microbiome of a cruise ship: ESKAPE bacteria are commonly found on surfaces and crew members' hands [version 2; peer review: 1 approved with reservations, 2 not approved]** Open Research Europe 2026, **5**:153 https://doi.org/10.12688/openreseurope.19710.2

First published: 05 Jun 2025, **5**:153 https://doi.org/10.12688/openreseurope.19710.1

REVISED **Amendments from Version 1**

In this revised version, the manuscript has been substantially clarified and reframed in response to reviewer feedback. The study is now explicitly presented as a cultivation-based, descriptive survey of viable bacteria rather than a comprehensive microbiome or ecological analysis. Throughout the text, claims regarding similarity between hand and surface samples have been revised to emphasize taxonomic overlap rather than statistical correlation, and interpretive language has been carefully aligned with the methodological limitations of the approach.

The Methods, Results, and Discussion sections have been expanded to more clearly describe the influence of culturing and DNA extraction on community composition, including the limited detection of fungi and viruses. The absence of negative and positive controls is now explicitly stated, and the scope and interpretation of antimicrobial resistance gene findings have been refined to reflect their exploratory nature. Overall, the revisions improve transparency, methodological clarity, and alignment between the study design, results, and conclusions.

Any further responses from the reviewers can be found at the end of the article

Key findings

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Introduction

During the past decade, next-generation sequencing technologies have fundamentally transformed metagenomics, making this type of research more affordable, which has led to an explosion of metagenomic studies.[1] Most microbiome studies focus on the human microbiome, particularly the gut microbiome (Figure 1). Environmental studies are emerging but remain less explored, just as the skin microbiome.

In this study, we focused on the microbiome of a cruise ship. In one sense, cruise ships are similar to other crowded environments where many people live or travel together, such as hotels, schools, or public transportation. Microbes found in built environments primarily originate from humans or enter from the outside environment through ventilation.[2] The Home Microbiome Project concluded that the home microbiome was largely derived from humans.[3]

A study on the microbiome of the Mexico City subway found a strong relationship between surface microbiomes and hand microbiomes.[4] The study described how passengers deposit bacteria onto surfaces and pick up others, making subway travel a means of microbial exchange.

On the other hand, cruise ships have some unique characteristics. Daily exposure to seawater and the salt mist that settles each night could significantly influence microbiome composition. Prado *et al.* studied the microbiome of a polar research

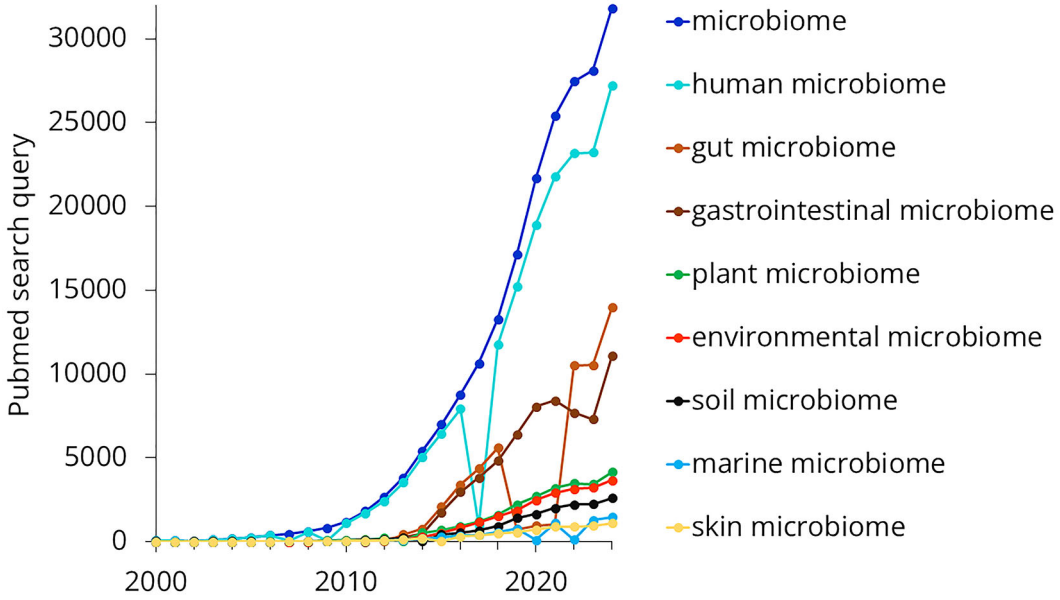

Figure 1. Trends in microbiome-related search terms on PubMed from 2000 to 2024.

ship,[5] finding that the predominant bacterial species were those typically associated with human skin. Their study also emphasized that human movement patterns had a major impact on the microbiome and noted that ship samples were consistent with those collected from other transportation facilities.

The aim of the present study was to map the composition of the microbiome found on a cruise ship. As previous studies have described a strong correlation between environmental microbiomes and skin microbiomes, the hand skin microbiome of crew members was also investigated.

Methods

We conducted a cross-sectional study aboard the *Celestyal Olympia*, a 12-deck cruise ship with a passenger capacity of 1,664 and a crew of 540. The primary objectives were (1) to characterize the microbial communities on frequently touched surfaces, (2) to examine the skin microbiome of crew members' fingertips, and (3) to compare the two microbial profiles for potential overlaps. Sampling was carried out using various agar-based methods, followed by DNA extraction and Nanopore long-read sequencing. Taxonomic classification was performed using Kraken2, and antibiotic resistance genes were identified via the CARD RGI tool. Below, we detail the logistics of sample collection, culture conditions, sequencing protocols, and subsequent bioinformatic analyses. Almost all samples were collected indoors on a closed ship environment operating at sea (except #A11, see later), where both temperature and humidity remained relatively stable.

The cruise ship

Sample collection was conducted onboard the *Celestyal Olympia*, a 12-deck cruise ship accommodating 1,664 passengers and 540 crew members traveling across the Aegean Sea. Passengers originated from all over the world and typically participated in 3- or 4-day voyages departing from and returning to Athens (Lavrio Port). From Monday to Friday, it followed the route marked with solid arrows, while from Friday to Monday, it skipped Rhodes to shorten the journey, as indicated by the dashed arrow (Figure 2).

Sample collection

Hand samples. Hand samples were collected from 19 volunteering participants during a hand hygiene training session organized for the crew. Volunteers were asked to gently touch a contact plate (VWR C31114TI) with their fingertips. These contact plates contained tryptic soy agar (TSA).

Surface samples. In total, 18 samples were collected from frequently touched surfaces aboard the ship, as such surfaces represent the highest risk for pathogen transmission. Four different agar media were used for surface sampling: TSA contact plates (VWR C31114TI), standard TSA plates (VWR 17114ZA), contact slides containing nutrient agar (NA) (VWR 535092Q), and MacConkey contact plates (Liofilchem S.r.l., Ref: 15329). Figure 3 illustrates the sampling



Figure 2. The cruise ship Celestyal Olympia (A), and its route during that season (B, C).

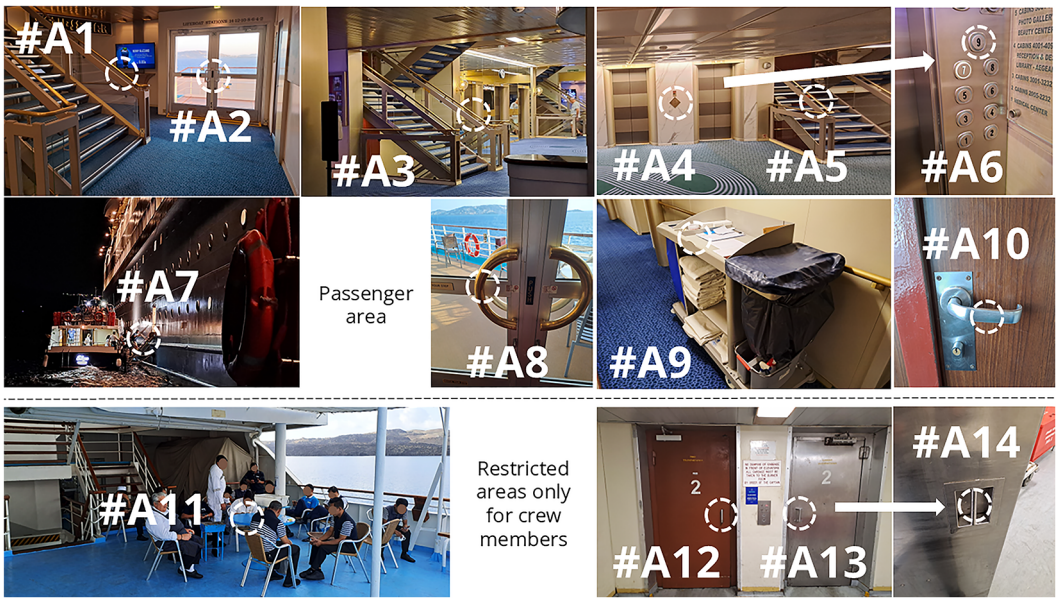

Figure 3. Sampling sites of surface samples collected using general-purpose agar plates.

sites where either nutrient agar (NA) or tryptic soy agar (TSA) was used. Both media are general-purpose and support the growth of most bacteria, providing an overview of the bacterial microbiome present on these surfaces. A detailed description of the sampling sites is provided in Table 1.

Four surfaces were sampled using MacConkey contact agar plates, a selective and differential culture medium designed to grow only Gram-negative bacterial species[6] (Table 1, #B1 – #B4).

Samples were incubated at 35–36°C for 48 hours. After incubation, all colonies from the agar were collected using a swab and stored in either Nucleic Acid Preservation Solution (NAPS NKS-40, Anthelos Ltd.) or sterile 0.9% NaCl solution (R-Biopharm Z0301).

Table 1. Detailed descriptions of the surfaces sampled during the study.

#	Collected by	Description of the sampling site
A1	TSA contact	Midship staircase rail on Deck 7, collected after the mandatory lifeboat drill, when all passengers were required to locate their lifeboat station, all situated on Deck 7.
A2	NA slide	Door handle on Deck 7, collected at the same time as sample #A1.
A3	TSA contact	Midship staircase rail on Deck 5, collected after the late-night show, which took place near this staircase.
A4	TSA contact	Outside panel of the elevator at the forward staircase, collected after passengers disembarked for excursions.
A5	TSA contact	Forward staircase rail, collected at the same time as sample #A4.
A6	TSA contact	Button #9 on the elevator panel inside one of the forward staircase elevators, collected in the afternoon after tea-time snacks were served on Deck 9.
A7	TSA contact	Rails of the mobile stairs used for docking tender boats (tender boats was used to transport passengers to the port when the cruise ship was too large to dock).
A8	TSA contact	Door handle at the Selena Lounge after the late-night Karaoke party. This door opened to an open deck, the only nearby smoking area.
A9	TSA contact	Cleaning trolley during the regular morning cleaning routine.
A10	NA slide	Restroom door knob on Deck 5, sampled during the late-night show. This restroom was located between the Muses Lounge (where the show took place) and the Argo Bar (with live music).
A11	NA slide	Crew table at the crew deck (Deck4). This were a crew-only are, where crew member had a chance to rest during their free time. The area was partially open, with the upper deck providing shading but lacking air conditioning, and the salty fog covered surfaces every night.
A12	NA slide	Outside handle of the food elevator in the crew-only area. This double elevator had a brown door for food transport, and a silver door for garbage. The food elevator was mainly used to transport food between Deck 4 kitchen, Deck 5 and Deck 9 restaurants.
A13	NA slide	Outside handle of the garbage elevator (silver door) in the same crew-only area. This elevator transported waste from all kitchens and restaurants to Deck 1 for disposal during port docking.
A14	TSA plate	Similar as #A13, but inside control panel of the same garbage elevator.
B1	MacConkey	Similar as #A14, inside panel of the garbage elevator, Button #3
B2	MacConkey	Black waterproof boots used by crew members handling waste, with the sample taken from the toe of a boot.
B3	MacConkey	Similar to #B2 but collected from the sole of the boot.
B4	MacConkey	Outside panel of the elevator in the kitchen on Deck 4.

Sequencing. For DNA extraction, the Invitrogen PureLink Microbiome DNA Purification Kit (Invitrogen) was used according to the manufacturer's protocol. Fragment lengths were measured using DNA ScreenTape on the TapeStation System (Agilent Technologies). Sequencing was conducted on a Nanopore MinION device with an R10 Flow Cell (FLO-FLG114). Sample preparation was performed using the Native Barcoding Kit 24 v14, following the SQK-NBD-114.24 protocol. Metagenomic data were uploaded to the following repository: European Nucleotide Archive (ENA) Accession number contains: Metagenomic data. Accession number: PRJEB87289. Link to the data: https://www.ebi.ac.uk/ena/browser/view/PRJEB87289.

Bioinformatics workflow. Taxonomic classification of metagenomic sequences was performed using Kraken2 with default parameters, followed by downstream analysis utilizing KrakenTools for data processing and visualization.[7,8] For antibiotic resistance analysis fastq file was converted to fasta format with bugaco sequence converter (https://sequence-conversion.bugaco.com/), then fasta files were uploaded to CARD RGI Resistance Gene Identifier (https://card.mcmaster.ca/analyze/rgi). All data were stored and subjected to further analysis using Microsoft Excel (Version 2308, Build 16.0.16731.20310).

Table 2. **Number of different species, genera, and phyla found in individual fingertip samples (P#1: Participant #1).**

	P#1	P#2	P#3	P#4	P#5	P#6	P#7	P#8	P#9	P#10	P#11	P#12	P#13	P#14	P#15	Total
Species	663	432	418	520	389	497	472	236	144	526	205	156	280	282	376	2495
Genus	296	168	157	204	147	155	180	100	42	213	86	53	85	100	151	749
Phylum	16	16	15	17	14	16	20	11	7	18	14	8	6	9	15	38

Table 3. **Proportions of superkingdoms in individual fingertip samples (percentage).**

Superkingdom	P#1	P#2	P#3	P#4	P#5	P#6	P#7	P#8	P#9	P#10	P#11	P#12	P#13	P#14	P#15	AVG
Viruses	0.00	0.02	0.00	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.00	0.05	0.02	0.01
Archea	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	99.88	99.95	99.81	99.95	99.68	99.92	99.93	99.94	99.95	99.94	99.94	99.96	99.98	99.75	99.95	99.90
Eukaryota	0.12	0.03	0.19	0.03	0.29	0.07	0.04	0.04	0.03	0.05	0.04	0.03	0.02	0.21	0.03	0.08

Table 4. **Proportions of the most prevalent bacterial phyla in individual fingertip samples (percentage).**

Phylum	P#1	P#2	P#3	P#4	P#5	P#6	P#7	P#8	P#9	P#10	P#11	P#12	P#13	P#14	P#15	AVG
Actinomycetota	3.27	43.20	22.36	22.24	11.20	7.33	35.90	33.50	2.48	5.52	5.01	4.86	37.80	6.36	12.97	16.93
Bacillota	94.87	56.29	76.41	77.09	87.04	88.97	63.06	66.07	93.22	66.12	94.40	93.68	42.39	87.26	84.34	78.08
Bacteroidota	0.26	0.03	0.04	0.01	0.01	0.05	0.01	0.01	0.02	0.02	0.02	0.00	0.00	0.03	0.01	0.04
Pseudomonadota	1.40	0.40	0.97	0.59	1.42	3.54	0.94	0.34	4.22	28.24	0.49	1.41	19.78	6.07	2.62	4.83
other	0.07	0.02	0.03	0.02	0.01	0.03	0.03	0.01	0.01	0.03	0.03	0.00	0.00	0.02	0.01	0.02

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Results

In total, 33 samples were loaded onto the flow cell, generating 10'369'710 reads. The number of reads per sample ranged from 16'245 to 3'509'833. The average fragment read length was 1'934 bae pairs, average Phred quality score was 33.0. Supplementary Table 1 contains data on read length and quality at the sample level. Neither negative nor positive control samples were measured.

Hand samples

Nineteen fingertip samples were collected. Due to insufficient DNA in some samples, only 15 samples were successfully sequenced. Table 2 summarizes the number of species, genera, and phyla identified in the samples. In total, 2'495 different species were detected across the 15 samples, representing 749 genera and 38 phyla.

The vast majority of the identified DNA fragments belonged to Bacteria (99.9%), as presented in Table 3. Viruses and Archaea were present in less than 0.02% of the samples. The only Eukaryota species detected in the samples was human, which represented the dead skin cells in the samples. No fungi were detected. Many fungi cannot grow on the agar plates used; however, the method was sensitive enough to detect human DNA. Therefore, the complete absence of fungi is most likely explained by the DNA extraction method, which was optimized for bacteria detection, and may not open fungal cell walls, to extract the DNA. The culturing process altered the microbial community composition, and as the results show, the extraction method likely failed to capture certain organisms. Due to this methodological bias, diversity indices and ecological distance metrics could not be reliably calculated. Instead, we report only the organisms that were detected in the samples. It should be noted, however, that this study provides information mainly on the bacterial component of the microbiome. Even this bacterial fraction may be represented in unrealistic proportions, as some bacteria that prefers our agar media may overgrow others, thereby distorting the relative abundances.

Among the bacteria, the most abundant phylum was Bacillota (78.08%), followed by Actinomycetota (16.93%), Pseudomonadota (4.83%), and Bacteroidota (0.4%) (Table 4). All other phyla represented less than 0.2% of the samples. At the phylum level, significant individual differences were observed. For example, Actinomycetota represented 43.20% of Participant #2's sample but only 2.48% in Participant #9's sample. Note that the samples also appeared to have different compositions at first glance (Figure 4).

At the genus level, each of the 15 samples exhibited a unique pattern (Table 5). In 14 cases, the most abundant genus was *Staphylococcus*, which, on average, accounted for 69.38% of the samples. However, in Participant #1's sample, the dominant genus was *Priestia* (87.78%), primarily composed of a single species, *Priestia flexa* (also known as *Bacillus flexus*).

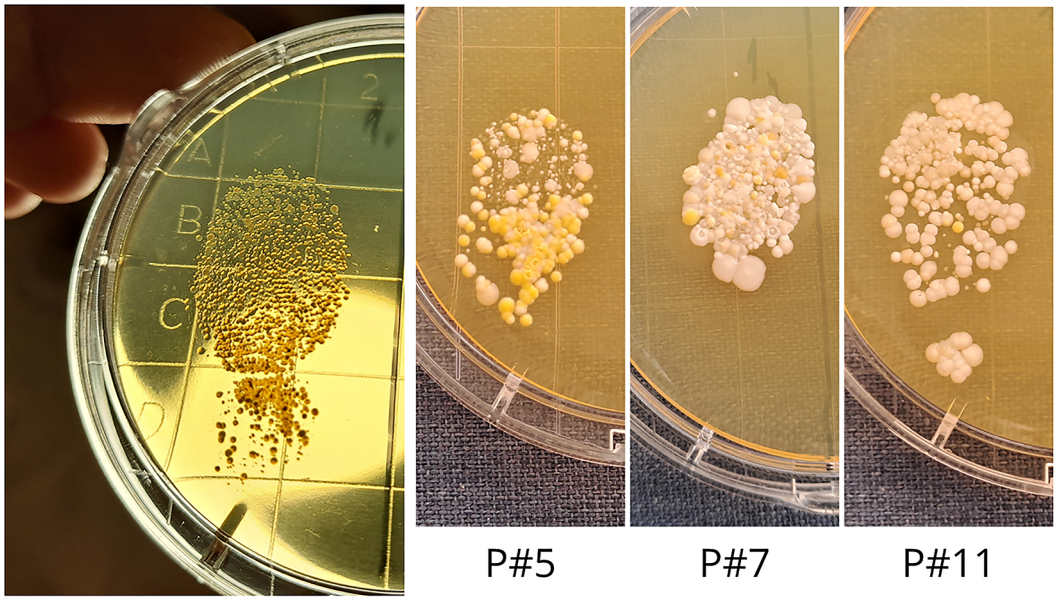

Figure 4. Individual differences of fingertip samples collected by TSA plates. (P#5: Participants #5).

Table 5. Top 19 most common genera in the hand samples, as percentage of each individual sample.

Genus	P#1	P#2	P#3	P#4	P#5	P#6	P#7	P#8	P#9	P#10	P#11	P#12	P#13	P#14	P#15	AVG
Staphylococcus	1.70	51.58	66.17	74.90	86.64	81.73	62.25	65.68	86.73	66.22	94.20	93.11	42.40	83.39	84.08	69.38
Micrococcus	2.04	31.20	18.35	18.22	10.47	4.48	33.81	31.36	0.91	4.07	2.94	4.13	36.88	1.59	11.57	14.13
Priestia	87.78	0.06	0.11	0.13	0.28	0.08	0.07	0.12	0.18	0.08	0.05	0.27	0.13	1.49	0.16	6.07
Pantoea	0.04	0.01	0.03	0.05	0.10	0.03	0.04	0.05	0.09	24.61	0.02	0.11	0.05	0.68	0.05	1.73
Rothia	1.19	9.59	0.10	3.14	0.54	1.44	0.22	0.10	1.49	0.90	0.22	0.39	0.14	2.72	0.19	1.49
Klebsiella	0.03	0.02	0.02	0.03	0.07	0.03	0.03	0.03	0.05	0.29	0.02	0.10	18.71	0.49	0.04	1.33
Bacillus	1.19	0.02	10.01	0.03	0.05	5.67	0.05	0.04	0.03	0.02	0.05	0.04	0.02	0.16	0.03	1.16
Corynebacterium	0.01	0.35	3.66	0.34	0.01	1.10	1.19	0.76	0.02	0.32	1.73	0.24	0.14	1.85	1.06	0.85
Mammaliicoccus	0.00	4.74	0.03	0.02	0.01	0.01	0.21	0.01	4.56	0.01	0.01	0.00	0.01	0.03	0.02	0.64
Streptococcus	0.02	0.00	0.01	2.11	0.01	0.78	0.07	0.00	0.40	0.09	0.02	0.19	0.00	1.95	0.00	0.38
Enterobacter	0.12	0.08	0.09	0.15	0.38	0.06	0.09	0.12	0.26	0.32	0.07	0.57	0.23	2.80	0.26	0.37
Moraxella	0.65	0.04	0.00	0.01	0.10	0.07	0.13	0.01	2.09	0.01	0.24	0.00	0.00	0.03	1.45	0.32
Escherichia	0.25	0.06	0.05	0.06	0.56	0.15	0.01	0.03	1.55	0.99	0.04	0.05	0.10	0.20	0.03	0.27
Neisseria	0.00	0.00	0.00	0.00	0.00	2.03	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.17
Agrobacterium	0.00	0.04	0.04	0.01	0.00	0.00	0.06	0.00	0.00	0.65	0.03	0.37	0.03	0.01	0.60	0.12
Kocuria	0.00	0.16	0.03	0.02	0.00	0.24	0.03	1.06	0.00	0.00	0.08	0.00	0.01	0.00	0.02	0.11
Exiguobacterium	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	1.27	0.00	0.00	0.01	0.00	0.04	0.00	0.09
Acinetobacter	0.08	0.02	0.03	0.05	0.10	0.04	0.02	0.03	0.09	0.05	0.02	0.08	0.04	0.52	0.08	0.08
Streptomyces	0.01	0.05	0.03	0.06	0.02	0.02	0.09	0.02	0.01	0.03	0.01	0.03	0.84	0.03	0.03	0.08

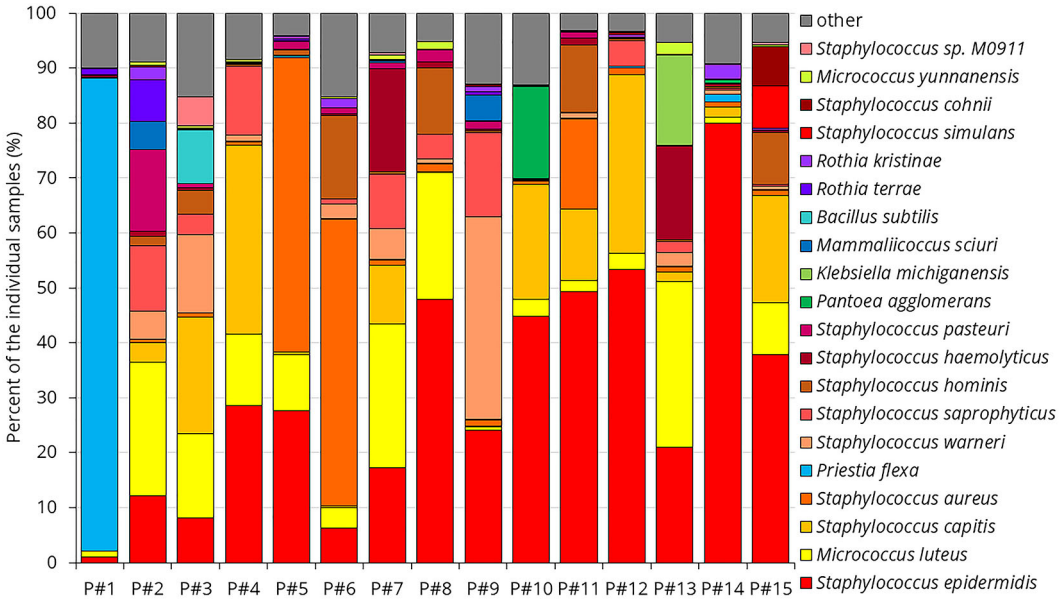

Figure 5. The 20 most common species in the individual hand samples.

This species has been described as both a urinary tract pathogen[9] and a marine bacterium.[10] The overrepresentation of *Priestia flexa* in Participant #1's sample suggests that it had the opportunity to proliferate somewhere. However, it remains unclear whether this proliferation occurred inside the body (as an infection or colonization) or it originated from the environment. No participants reported any ongoing infections, although, they were not directly asked about that.

Figure 5 illustrates the most common species detected in the hand samples. Individual samples display significant variability, with no two samples being identical. Notably, the top 20 species account for 84.77–96.80% of the total sample composition. Among these, 11 species belong to the genus *Staphylococcus.*

Table 5 highlights that the *Staphylococcus* genus constitutes the vast majority of the samples. Figure 5 further demonstrates that various *Staphylococcus* species occur in widely different proportions across the samples. Table 6 summarizes the *Staphylococcus* species alongside the *Staphylococcus* viruses identified in the hand samples.

The host of *Staphylococcus* phage phiRS7 is reported to be *S. saprophyticus*.[11] *S. saprophyticus* was most prominently detected in the hand sample of Participant #9 (15.49%), where the highest number of phiRS7 phages was also found. Similarly, phage StB27's host is identified as *S. hominis.* In the two samples where these phages were detected (Participants #6 and #11), the largest proportions of *S. hominis* were also observed (15.09% and 12.24%, respectively). Interestingly, Participant #8 also exhibited a substantial proportion of *S. hominis* (12.12%), but the StB27 phage was absent. Phage StB12, detected in the hand sample of Participant #11, also uses *S. hominis* as its host. In this case, 12.24% of *S. hominis* appeared sufficient to support the presence of two distinct phages.

Surface samples

A total of 14 surface samples were collected using general-purpose media (NA and TSA) from various surfaces (Figure 3). Some samples were dominated by a single species, what is probably a bias caused by the culturing step. For example, 94% of Sample #A8 consisted of *Rothia kristinae*, while 90% of Sample #A6 was composed of *Pantoea agglomerans.* This can be attributed to the fact that in surface samples, in some cases only a few colonies grew (in contrast with the hand samples, hundreds of colonies were consistently observed in all samples.) All surface samples contained a high number of species, ranging from 184 in Sample #A6 to 4'320 species in Sample #A11. Table 7 summarizes the species that were found in all, or nearly all (13 out of 14) surface samples and compares their prevalence to that in the hand samples.

A total of 22 species were found in all 14 surface samples, and 17 species were detected in 13 out of 14 samples. These species were also highly prevalent in hand samples. Notably, the top 10 most common hand-associated bacteria were found in all surface samples. All surface samples contained human DNA, likely representing dead skin cells. Only four

Table 6. **Top 30 most common *Staphylococcus* species and all *Staphylococcus* phages detected in the hand samples.**

Staphylococcus species	P#1	P#2	P#3	P#4	P#5	P#6	P#7	P#8	P#9	P#10	P#11	P#12	P#13	P#14	P#15	AVG
Staphylococcus epidermidis	1.02	12.16	8.20	28.52	27.65	6.31	17.26	47.89	24.09	44.83	49.27	53.30	20.96	79.91	37.89	30.62
Staphylococcus capitis	0.04	3.44	21.22	34.40	0.46	0.20	10.67	0.10	0.15	20.88	13.06	32.49	1.75	1.88	19.55	10.69
Staphylococcus aureus	0.04	0.65	0.75	0.71	53.54	52.26	1.00	1.46	1.09	0.71	16.46	1.23	0.88	0.87	0.91	8.84
Staphylococcus warneri	0.01	5.13	14.22	1.08	0.13	2.77	5.71	0.74	36.83	0.06	1.04	0.11	2.57	0.73	0.58	4.78
Staphylococcus saprophyticus	0.03	11.89	3.66	12.55	0.08	0.89	9.78	4.57	15.43	0.03	0.08	4.56	1.88	0.27	0.31	4.40
Staphylococcus hominis	0.22	1.72	4.39	0.26	0.88	15.09	0.56	12.12	0.20	0.11	12.24	0.32	0.30	0.40	9.40	3.88
Staphylococcus haemolyticus	0.26	0.82	0.38	0.12	0.15	0.34	18.75	1.07	0.35	0.13	1.22	0.19	17.11	0.41	0.46	2.78
Staphylococcus pasteuri	0.01	14.98	0.87	0.05	1.52	1.09	1.07	2.11	1.43	0.05	1.05	0.05	0.03	0.14	0.07	1.64
Staphylococcus simulans	0.00	0.03	0.14	0.06	0.05	0.03	0.04	0.03	0.05	0.14	0.04	0.37	0.02	0.07	7.74	0.59
Staphylococcus cohnii	0.00	0.28	0.06	0.02	0.02	0.04	0.09	0.01	0.35	0.01	0.11	0.20	0.01	0.02	7.17	0.56
Staphylococcus sp. M0911	0.00	0.01	5.37	0.00	0.01	0.01	0.47	0.01	-	-	0.00	-	0.00	-	0.52	0.43
Staphylococcus sp. 11-B-312	0.01	1.71	0.01	0.01	0.02	0.01	0.20	0.01	2.37	0.01	0.00	0.62	0.01	0.01	0.02	0.33
Staphylococcus sp. SB1-57	0.00	0.01	4.19	0.00	0.00	0.00	0.06	-	0.01	0.00	0.01	0.01	0.01	0.01	0.22	0.30
Staphylococcus caprae	-	0.06	1.59	0.05	0.04	0.02	0.06	0.02	2.08	0.02	0.03	0.01	0.02	0.01	0.05	0.27
Staphylococcus ureilyticus	0.01	0.81	0.02	0.01	0.01	0.01	0.27	0.01	1.43	0.01	-	0.28	0.00	0.03	0.02	0.19
Staphylococcus lugdunensis	-	0.03	0.22	0.06	0.08	0.04	0.14	0.11	0.22	0.04	0.09	0.08	0.06	0.04	0.07	0.08
Staphylococcus pettenkoferi	0.00	0.84	0.07	0.05	0.02	0.01	0.00	-	0.17	0.01	0.01	0.02	0.01	0.02	0.01	0.08
Staphylococcus equorum	0.00	0.12	0.11	0.15	0.01	0.02	0.06	0.09	0.11	0.01	0.01	0.05	0.03	0.02	0.09	0.06

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Table 6. *Continued*

Staphylococcus species	**P#1**	**P#2**	**P#3**	**P#4**	**P#5**	**P#6**	**P#7**	**P#8**	**P#9**	**P#10**	**P#11**	**P#12**	**P#13**	**P#14**	**P#15**	**AVG**
Staphylococcus pseudintermedius	0.01	0.05	0.03	0.06	0.04	0.05	0.10	0.05	0.11	0.03	0.04	0.06	0.05	0.02	0.06	0.05
Staphylococcus argenteus	-	0.00	0.01	0.00	0.30	0.26	0.02	-	-	0.00	0.02	-	0.00	-	0.01	0.04
Staphylococcus xylosus	-	0.04	0.08	0.05	0.03	0.06	0.05	0.10	0.04	0.01	0.02	0.01	0.03	0.02	0.04	0.04
Staphylococcus arlettae	-	0.02	0.14	0.08	0.02	0.03	0.03	0.06	0.05	0.00	0.01	0.02	0.01	0.03	0.04	0.04
Staphylococcus schweitzeri	-	-	0.01	-	0.24	0.20	0.01	-	-	-	0.01	-	-	0.01	-	0.03
Staphylococcus petrasii	0.00	0.07	0.02	0.02	0.00	0.01	0.03	0.01	0.04	0.01	0.01	0.06	0.04	-	0.02	0.02
Staphylococcus simiae	0.00	0.00	0.05	0.01	0.10	0.14	0.01	-	0.01	0.00	0.01	0.00	0.00	-	0.01	0.02
Staphylococcus succinus	-	0.02	0.05	0.02	0.01	0.02	0.01	0.01	0.10	0.00	0.00	0.00	0.00	0.01	0.02	0.02
Staphylococcus nepalensis	-	0.02	0.07	0.00	0.01	0.01	0.02	0.03	0.02	0.01	0.00	0.01	0.00	-	0.04	0.02
Staphylococcus carnosus	0.00	0.03	0.04	0.01	0.00	0.01	0.02	0.03	0.04	0.00	0.00	0.01	0.00	0.06	0.01	0.02
Staphylococcus sp. NRL 16/872	-	0.01	0.13	0.01	-	-	0.02	0.00	-	0.00	0.00	0.00	0.02	0.01	0.00	0.01
Staphylococcus gallinarum	-	0.01	0.14	0.01	0.01	0.00	0.01	-	0.02	0.00	-	-	0.00	-	0.01	0.01
other *Staphylococcus*	0.01	0.10	0.68	0.13	0.30	0.37	0.23	0.20	0.21	0.04	0.14	0.11	0.06	0.17	0.23	0.20
***Staphylococcus* phages**	**P#1**	**P#2**	**P#3**	**P#4**	**P#5**	**P#6**	**P#7**	**P#8**	**P#9**	**P#10**	**P#11**	**P#12**	**P#13**	**P#14**	**P#15**	**AVG**
Staphylococcus phage phiRS7	-	0.005	-	0.007	-	-	0.014	-	0.018	-	-	0.005	0.002	-	0.003	0.004
Staphylococcus phage StB27	-	-	-	-	-	0.005	-	-	-	-	0.011	-	-	-	0.001	0.001
Staphylococcus phage StB12	-	-	-	-	0.001	-	-	-	-	-	0.004	-	-	-	0.002	0.000
Staphylococcus phage StB20-like	-	-	-	-	-	-	0.001	-	-	-	-	-	-	-	-	0.000
Staphylococcus phage DW2	-	-	-	-	0.001	-	-	-	-	-	-	-	-	-	-	0.000

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Table 7. Summary of species prevalence across surface samples. Color-codes indicates frequency in hand samples.

Founded in 14/14 samples	Founded in 13/14 samples	Color code
Acinetobacter baumannii	*Acinetobacter pittii*	top1-10 in hand samples
Cytobacillus kochii	*Bacillus altitudinis*	top11-20 in hand samples
Enterobacter asburiae	*Bacillus safensis*	top21-30 in hand samples
Enterobacter hormaechei	*Bacillus subtilis*	top31-50 in hand samples
Enterobacter roggenkampii	*Bacillus velezensis*	top51-100 in hand samples
Escherichia coli	*Enterobacter kobei*	
Homo sapiens	*Enterobacter mori*	
Klebsiella pneumoniae	*Klebsiella quasipneumoniae*	
Micrococcus luteus	*Mammaliicoccus sciuri*	
Niallia circulans	*Micrococcus sp. KBS0714*	
Pantoea agglomerans	*Micrococcus yunnanensis*	
Priestia flexa	*Moraxella osloensis*	
Priestia megaterium	*Rothia terrae*	
Rothia kristinae	*Staphylococcus cohnii*	
Staphylococcus aureus	*Staphylococcus pettenkoferi*	
Staphylococcus capitis	*Staphylococcus sp. 11-B-312*	
Staphylococcus epidermidis	*Staphylococcus ureilyticus*	
Staphylococcus haemolyticus		
Staphylococcus hominis		
Staphylococcus pasteuri		
Staphylococcus saprophyticus		
Staphylococcus warneri		

species common on surfaces were not among the top 100 most prevalent species in hand samples; *Cytobacillus kochii* (#112 in hand samples), *Enterobacter mori* (#118), *Bacillus safensis* (#134) and *Acinetobacter pittii* (#152). These findings suggest a strong correlation between the microbiome of the surfaces and the microbiome of human hands in this environment. This emphasizes the role of surfaces in the potential transmission of bacteria, and thus, infection-transmission.

In healthcare settings, ESKAPE bacteria are often discussed due to their association with life-threatening, healthcare-associated infections. These bacteria are known for their ability to "escape" the effects of antibiotics by developping resistance. The acronym ESKAPE stands for: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp,* and sometimes *Escherichia coli* is also mentioned as an ESKAPE bacteria.[12] Five of the seven ESKAPE bacteria (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and three different *Enterobacter* species and *Escherichia coli*) were present on all surfaces. Instead of the fact that in the cruise ship everyone was healthy, or at least felt themselves good enough to participate a vacation trip. These findings underscore why some of the healthcare-infections cannot be prevented; bacteria often arrive to the healthcare facility together with the patients This highlights the critical need for continuous and thorough disinfection of surfaces, particularly in environments with vulnerable individuals.

The samples analyzed so far were predominantly dominated by *Staphylococcus* and other Gram-positive species. In the case of hand samples, 18 of the top 20 bacteria were Gram-positive, while only 2 were Gram-negative: *Pantoea agglomerans* and *Klebsiella michiganensis.* Similarly, among the species found in all surface samples, Gram-positive bacteria were dominant, with 14 species detected, compared to only 7 Gram-negative species, including *Acinetobacter baumannii*, various *Enterobacter* species, *Klebsiella pneumoniae*, and *Pantoea agglomerans.* Notably, all the Gram-negative bacteria mentioned here were known pathogens, with some classified among the ESKAPE pathogens. For instance, *Pantoea agglomerans* has been associated with wound infections[13] and pneumonia in immunocompromised individuals.[14] Meanwhile, *Klebsiella michiganensis* is an emerging human pathogen.[15]

To further investigate the Gram-negative bacterial population on surfaces, some samples were collected using MacConkey agar plates (Table 1). These samples were mostly targeted areas likely to be the most contaminated on the cruise ship, including the elevator used by the crew to transport waste and the waterproof boots worn by crew members working in the waste disposal area (Samples #B1, #B2, and #B3, Figure 6).

Figure 7 shows the most commonly identified bacteria in each sample. Sample #B1 was dominated by *Acinetobacter* species, with 7 different *Acinetobacter* species ranked among the top 21 species. Together, these *Acinetobacter* species accounted for 64.70% of the total sample. In sample #B2, *Klebsiella* species were more prevalent, while sample #B3 was dominated by *Enterobacter* species. *Lactiplantibacillus carotarum* was first isolated in 2023 from fermented carrot juice.[16] *Ligilactobacillus salivarius* is a lactic acid bacterium, recognized as a promising probiotic, and interestingly, it is a Gram-positive species.[17]



Figure 6. Environmental surface sampling using MacConkey contact agar plates at the waste disposal area.

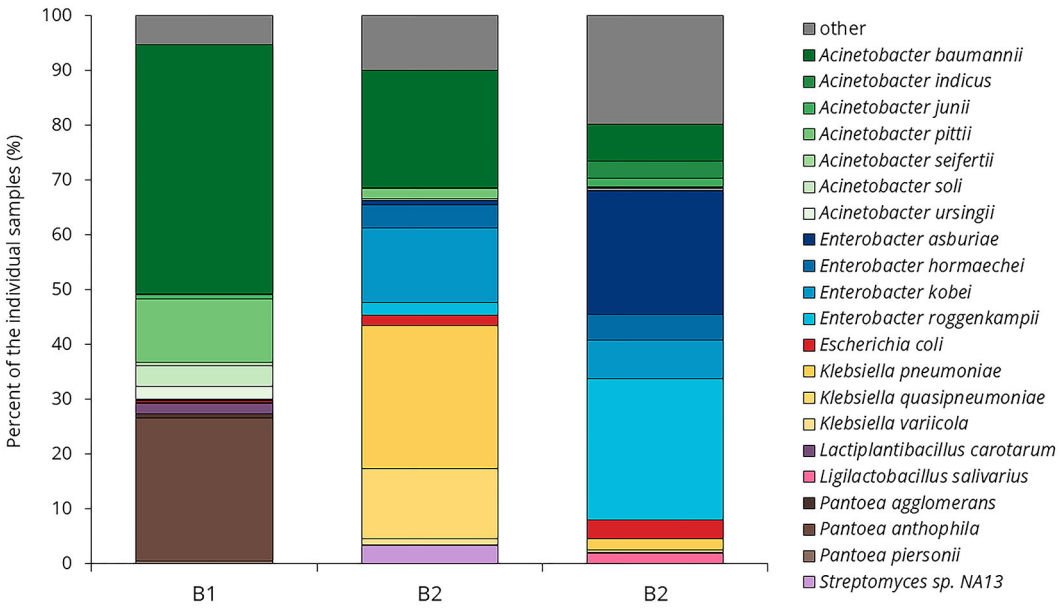

Figure 7. Bacterial profiles from waste disposal samples on MacConkey agar.



Genus	Presence in #B4 sample (%)
Enterobacter	82.74
Klebsiella	4.03
Acinetobacter	3.55
Pantoea	3.32
Serratia	1.64
Nostoc	1.48
Escherichia	0.97
Leclercia	0.67
Citrobacter	0.26
Staphylococcus	0.23
Rhizobium	0.20
Salmonella	0.14
Pseudomonas	0.13
Pluralibacter	0.13
Priestia	0.07
Raoultella	0.07
Pectobacterium	0.03
Buttiauxella	0.02
Micrococcus	0.02
Cronobacter	0.02
other	0.28

Figure 8. Sample collected in the main kitchen using a MacConkey contact agar plate.

The final sample (#B4) differed from the others, as it was collected from the elevator control panel in the main kitchen area (Table 1, Figure 8). Here, fewer Gram-negative bacteria were expected due to regular disinfection. On the other hand, the site was also frequently touched. In sample #B4, 1'501 species were identified, which was surprisingly high compared to the other samples: 661 species in #B1, 1'294 species in #B2, and 1'661 species in #B3, respectively.

Sample #B4 was dominated by *Enterobacter* species, with a total of 82.74% of the sample belonging to this genus. The most prevalent species were *Enterobacter hormaechei* (36.56%), *Enterobacter cloacae* (19.30%), *Enterobacter roggenkampii* (7.29%), *Enterobacter mori* (5.42%), and *Enterobacter asburiae* (2.77%). Among the top 20 species, six additional Enterobacter species were identified.

Other known pathogens were also found among the top 20 species, including *Acinetobacter baumannii*, *A. pittii*, *Klebsiella pneumoniae*, *K. michiganensis*, *Escherichia coli*, and *Serratia marcescens.* The only two non-pathogenic species in the top 20 list were *Nostoc sp. C057*, a cyanobacterium, and *Rhizobium sp. C104*, a soil bacterium that lives in endosymbiosis with plant roots and plays an important role in nitrogen fixation.[18] The presence of soil bacteria suggests that some of these microbes may have entered the kitchen through vegetables being processed.

To better assess the potential risks posed by these bacteria, resistance genes were analyzed in sample #B4. Many antibiotic resistance genes were present in the sample, with varying frequencies. In Table 8, the identified resistance genes were categorized by gene families and their frequency of occurrence.

The two most common resistance gene families were MSF and RND antibiotic efflux pumps. Some resistance genes were directly linked to ESKAPE bacteria, including *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Staphylococcus aureus.* Additionally, *Shigella flexneri* was also identified. *S. flexneri* is an emerging pathogen that causes diarrhea.[19] It was present on 4 out of 15 (27%) hands and on 8 out of 18 (44%) surfaces.

As mentioned earlier, the culturing step introduced substantial bias, as some viable bacteria outgrew others. For this reason, we did not normalize the CARD ARG results to read counts; instead, we aimed to highlight how many resistance genes could be detected in a theoretically low-risk environment.

Figure 9 compares the composition of the hand samples and surface samples collected using general-purpose agar plates. *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Mammaliicoccus*, and *Klebsiella* were more dominant in hand samples than in surface samples. The first four genera are Gram-positive bacteria, their proportion likely decreased due to their limited survival capability on surfaces. In contrast, *Klebsiella* is a Gram-negative bacterium that can survive on surfaces for days to more than a month, so this difference cannot be explained by survival capability.[20] Conducting statistical

Table 8. **Antimicrobial resistance genes found in sample #B4, grouped by their frequency of occurrence.**

AMR gene family	ARO Term		
	Rare (Founded 1–5 times)	Moderate (Founded 6–40 times)	Common (Founded 41+ times)
ACT beta-lactamase	ACT-17, ACT-47, ACT-47, ACT-86, ACT-118, ACT-179, ACT-192		
ANT(3',',)	ANT(3")-IIa		
BlaZ beta-lactamase	PC1		
elfamycin resistant EF-Tu	*Escherichia coli* EF-Tu mutants		
fosfomycin thiol transferase	fosA5		FosA2
major facilitator superfamily (MFS) antibiotic efflux pump	emrB, *Staphylococcus aureus* norA	*Klebsiella pneumoniae* KpnH, leuO, H-NS	emrR
MIR beta-lactamase	MIR-35		
pmr phosphoethanolamine transferase	ArnT		
resistance-nodulation-cell division (RND) antibiotic efflux pump	*Enterobacter cloacae* acrA, *Shigella flexneri* acrA, ramA	baeR, baeS, CRP, oqxA, marA	*Escherichia coli* AcrAB-TolC with MarR mutations, rsmA
small multidrug resistance (SMR) antibiotic efflux pump	abeS		*Klebsiella pneumoniae* KpnE, *Klebsiella pneumoniae* KpnF
Target protecting FusB-type protein conferring resistance to Fusidic acid	fusB		

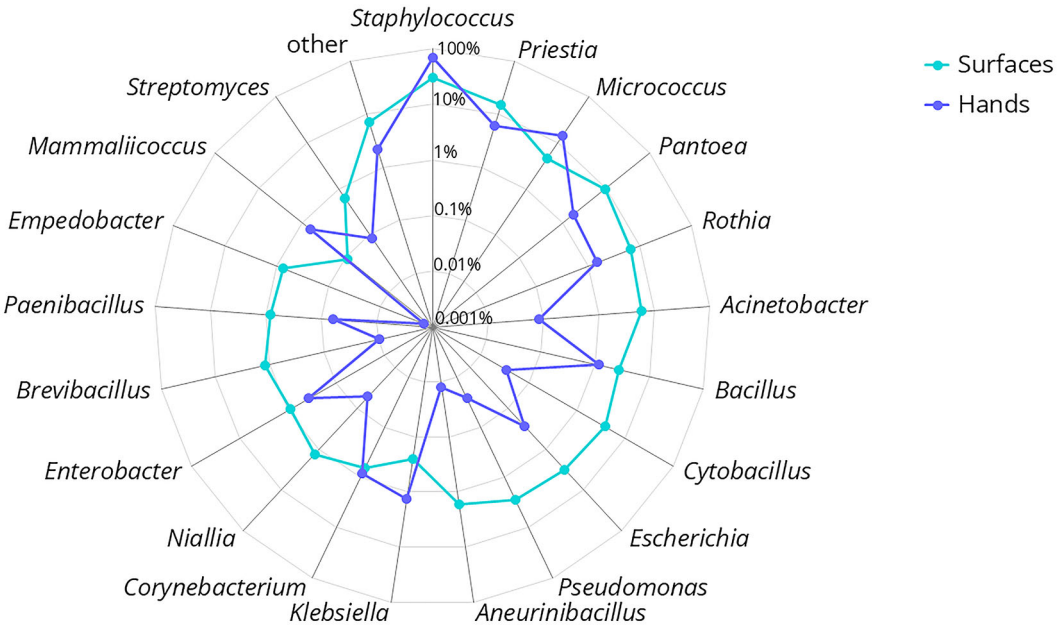

Figure 9. **Comparison of the composition of surface and hand samples for the 20 most common genera.**

analyses on a dataset that is clearly biased by culturing and extraction limitations would not be appropriate, but Figure 9 suggest a correlation.

Discussion

Our study is more a descriptive microbiological survey than a full-scale microbiome analysis. The composition of hand microbiome samples was consistent with the literature, being dominated by *Staphylococcus* species.[21] In our samples, we observed *Corynebacterium* species less frequently than reported in the literature, where they are cited as one of the three most abundant bacterial genera.[22]

Individual hand samples were quite variable, despite the fact that crew members lived onboard 24/7, shared meals, and spent their days in the same relatively small area. In the case of this specific ship, the season started in mid-March and ended in mid-November. Most of the crew spent the entire season onboard, without any days off. Fingertip sampling was carried out between October 30th and November 2nd, when the crew had been living together for the past 7–8 months. The differences in the composition of the hand microbiomes were remarkable. This study only recorded a snapshot of the microbiomes, so we cannot answer the question of how stable these individual compositions were.

As mentioned previously, our analysis appeared to be blind to fungi, as our DNA extraction method was not capable of breaking the fungal cell wall. A previous review states that human skin contains a diverse fungal microbiome.[21] In a study on a polar research ship, bacteria formed only 83.7% of the surface samples, with the second largest domain being eukaryotes (16.2%), includes fungi.[5] Another review claims that hand samples also contain one-quarter viruses.[23] Based on this, it seems that our method was also unsuitable for detecting viruses, as we detected less than 0.01% viruses in our hand samples. This suggests that from this analysis, we can only draw conclusions regarding the bacterial portion of the microbiome.

Before shifting focus from hand microbiomes to surface-associated communities, it is important to underscore the dynamic interplay that likely exists between these two bacterial reservoirs. As our data show, the composition of hand microbiomes can be highly individualized, yet the strong overlap with surface microbes suggests frequent bidirectional transfer. Hands may deposit skin-associated bacteria onto shared surfaces, which can in turn become ecological "hotspots" for microbial persistence and redistribution. This interplay underscores the value of examining both hand and surface microbiomes in tandem when assessing the potential for pathogen spread or environmental colonization in communal settings, such as a cruise ship.

ESKAPE bacteria were frequently described in both hand and surface samples. Resistance genes were also present in large numbers in the analyzed sample (#B4). On one hand, it is concerning that so many potential pathogens were found on an otherwise clean-looking, regularly disinfected surfaces. On the other hand, these pathogens were also present on hands, suggesting that we can expect them on every surface touched by people.

The main limitation of our study is that all of our sampling involved some culturing steps. Culture-based methods may bias community composition. As mentioned, we were able to detect human DNA in all samples, which indicates that, despite the culturing effect, we were still able to detect small amounts of non-dividing DNA. We made sure to be very cautious in interpreting the results, disregarding the very low percentage of detections, as we did not want to draw incorrect conclusions from possible misclassification. From these data, we could not calculate diversity indices. Within these methodological constraints, we focused on the conclusions that could reasonably be drawn: numerous antimicrobial resistance genes were detected in an environment where healthy individuals spend their holiday. We consider this observation potentially valuable from an infection control perspective, although additional, better-controlled experiments are clearly required.

Conclusions

Our findings describe a strong overlap between the cruise ship surface microbiome and that of crew members' hands. While it remains unclear whether this similarity is driven by the environment's impact on the hand microbiome or vice versa, our results suggest that human-associated microbes are likely deposited onto surfaces despite rigorous cleaning regimens. This highlights the role of these surfaces in spreading potential pathogens.

Importantly, we detected five of the seven ESKAPE bacteria on virtually all sampled surfaces. In hospital environments, it has been documented that surfaces are often contaminated with pathogens,[20] but this seems to be a more general phenomenon. These pathogens may be more common in our surroundings than we previously thought. They can pose a serious threat in hospitals, not only because they easily develop antibiotic resistance but also because they can be found everywhere, continuously spread by patients, staff members, and visitors. We acknowledge that our study represents only

a first step in addressing this topic and has several methodological limitations. Nevertheless, we believe that these findings are important from an infection control perspective and can add a different view to our knowledge about the spread of ESKAPE bacteria and antimicrobial resistance.

Moreover, although we cannot definitively assess the influence of environmental factors such as salt-laden air, frequent disinfection protocols, or air conditioning on microbial assemblages, these variables likely shape bacterial survival and transmission. Understanding how such factors interact with the human microbiome in enclosed, high-traffic settings is critical for informing more effective cleaning strategies and mitigating the spread of clinically relevant pathogens in both healthcare and non-clinical environments.

Ethics and consent

As the project coordinator (University of Thessaly) is based in Greece and the work package leader (University of South-Eastern Norway) is Norwegian, the research proposal was submitted to both the Greek and Norwegian ethical boards. The Research Ethics Committee of the University of Thessaly approved the project (decision number: 59/19.09.2022, date: Sep 19, 2022). The proposal was submitted to the Norwegian Medical Research Ethics Committee (REK), which determined that the project falls outside the scope of the Health Research Act (i.e., it is not considered a clinical study) and therefore does not require ethical approval (reference number: 562942, date: Jan 30, 2023). The Norwegian Agency for Shared Services in Education and Research (Sikt) was notified of the project and granted approval (reference numbers: 164497, date: Oct 15, 2023, and 664058, date: Mar 6, 2024).

This study did not involve human participants, human samples, or personal data. The microbiome samples analyzed were not associated with any individuals. The hand hygiene training, where fingertip samples were collected was part of an intervention study. All crew members who participated in the training provided written consent. The study was conducted in accordance with general ethical standards aligned with the Declaration of Helsinki.

Data availability

The metagenomic data is publicly accessible. *Repository:* European Nucleotide Archive (ENA): *Accession number contains:* Metagenomic data. *Accession number:* PRJEB87289. *Link to the data:* https://www.ebi.ac.uk/ena/browser/view/PRJEB87289.

Extended data

The extended data underlying this article are available on

Figshare:

https://doi.org/10.23642/usn.29063267.v1[24]

This project contains the following extended data:

- Supplementary Table 1
- Annex 3
- Consent Sample
- Information letters

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgements

We thank all crew members of *Celestial Olympia* for supporting the study.

HEALTHY SAILING project has received funding from the European Union's Horizon Europe Framework Programme (HORIZON) under Grant Agreement number 101069764. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Climate, Infrastructure and Environment Executive Agency (CINEA). Neither the European Union nor the granting authority can be held responsible for them. This work was funded by UK Research and Innovation (UKRI) under

the UK government's Horizon Europe funding guarantee [grant number 10040786], [grant number 10040720]. This work has received funding from the Swiss State Secretariat for Education, Research and Innovation (SERI).

Sequencing was conducted in a laboratory supported by the Lendület (Momentum) Programme of the Hungarian Academy of Sciences, Grant number: LP2020-8/2020 to DT.

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Current Peer Review Status: ✗ ✗ ?

Version 2

Reviewer Report 09 April 2026

https://doi.org/10.21956/openreseurope.25056.r71556

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Lucyna Gozdzielewska
Glasgow Caledonian University, Glasgow, UK

Summary
This is an interesting cross-sectional study describing the microbial composition of high-touch environmental surfaces on a single cruise ship, as well as on the hands of crew members. It is evident that the authors made substantial revisions following previous feedback. I note that the methodological concerns previously raised in relation to microbiological and sequencing approaches (e.g. culturing bias, lack of diversity or community analyses, and absence of controls) appear to have been addressed, and the study is now presented more appropriately as a descriptive microbiological survey rather than a comprehensive microbiome study. As my expertise is not in microbiology or metagenomic methods, I am unable to comment on these aspects in detail; however, the revisions made in response to the previous reviewers' comments in this area appear reasonable. However, some minor issues remain, as detailed below.

Methods section
The authors state that samples were taken indoors where temperature and humidity remain relatively stable. However, in their response to previous feedback, they indicate that these variables were not measured. As such, this statement appears to be an assumption. I think this should be clarified, or removed from the paper.
The authors report that hand samples were collected during hand hygiene training sessions. It would be helpful to clarify whether crew members performed hand hygiene during these sessions, and whether samples were taken before or after training, as hand hygiene could substantially influence microbial load on hands.
Four different agar media were used for surface sampling, with different media applied to different sites. It is unclear, however, why specific media were chosen for each site, and the criteria for these decisions should be clarified.

Discussion & conclusion
I think the paper could be improved by explicitly identifying limitations, including the study being conducted on a single cruise ship, with sample size of both environmental and hand samples being fairly small, which limits generalisability and sample representativeness.

The discussion (and conclusion) section refers to healthcare-associated infections and microbial species commonly found in healthcare settings, including ESKAPE organisms. It would be helpful if the authors clarified the relevance of these references, since the risks in clinical environments with susceptible patients differ from those on a cruise ship, and to ensure that the interpretation of the findings is appropriately contextualised for the cruise ship setting. I believe this would enhance the academic merit of the paper and could help develop more meaningful conclusions.
The conclusions mention environmental factors that may influence microbial survival and transmission, including disinfection protocols and air conditioning. I am wondering if the authors have also considered the potential role of water systems on the cruise ship. Evidence from healthcare settings suggests that pipes, taps, sinks and showers can act as reservoirs for microorganisms, including biofilms and antimicrobial-resistant organisms, and may contribute to transmission. It might be useful to briefly consider whether water systems could become contaminated and similarly influence microbial composition or pathogen spread in this setting.

In addition, Ethics and consent section states “This study did not involve human participants, human samples, or personal data”, which I find misleading as samples were collected from the hands of crew members.

Thank you for the opportunity to review this manuscript. I wish the authors all the best with their revisions and the further development of this work.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hand hygiene, Infection prevention and Control, nursing

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 01 December 2025

https://doi.org/10.21956/openreseurope.21320.r64218

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Marja Roslund
Natural Resources Institute in Finland, Helsinki, Finland

This study offers an initial characterization of the microbial communities present on frequently touched surfaces aboard a cruise ship, as well as on the fingertips of crew members. Using a combination of cultivation-based sampling and Nanopore long-read metagenomic sequencing, the authors provide exploratory data relevant to infection control in a confined maritime environment.
While the growing media support the growth of many bacterial taxa, cultivation-based sampling inherently captures only a subset of the microbial community. This methodological choice should be explicitly acknowledged as a major limitation.
Major methodological and analytical limitations
Several essential analytical components of metagenomic research are missing from the study:

- **No diversity analyses were performed.** Alpha diversity, beta diversity, and ecological distance metrics are standard tools to compare microbial communities. Their absence limits the ability to rigorously assess similarities or differences between surface and hand microbiomes.
- **No ordination analyses were conducted.** Principal coordinates analysis or other ordination methods are necessary to visually and statistically support claims of microbiome similarity.
- **No statistical tests were used.** Without statistical measures, the authors cannot substantiate statements about similarities or differences between sample groups.

Concerns about controls
The manuscript does not clearly state whether negative controls (e.g., blank extraction controls, field controls) or positive controls (e.g., a mock community) were included. These controls are essential in metagenomic studies to detect contamination, validate extraction and sequencing performance, and calibrate classification accuracy.
Without them, it is difficult to differentiate true biological signal from potential laboratory or environmental contaminants.
Generalizability and overall limitations
In addition to the methodological gaps, the study remains limited by:

1. **A small sample size**, especially for hand samples.
2. **Sampling from a single ship**, preventing generalization to cruise ships more broadly.
3. **Cultivation-based sampling**, which excludes non-culturable microorganisms.
4. **Reliance on a sequencing method with known accuracy constraints.**

Given these limitations, **strong conclusions cannot be drawn**, and statements implying broad

generality or high confidence, such as *"Our findings reveal a strong overlap between the cruise ship surface microbiome and that of crew members' hands"*,should be rewritten to reflect the exploratory nature of the work.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology, Environmental Ecology, Environmental Health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 22 November 2025

https://doi.org/10.21956/openreseurope.21320.r61646

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Hesham Amin
University of Bergen, Bergen, Norway

Overall comment

rThis is an interesting study and provide valuable data on cruise ship surface and hand microbiomes using Nanopore sequencing. The topic is relevant, especially after COVID-19. However, the study is methodologically limited, analytically underdeveloped, and overstretched in

several parts. It reads more like a descriptive microbiological survey than a true microbiome study. The culturing step and lack of quantitative or ecological metrics weaken its scientific aim of the study.
Strengths including

1. New study environment as cruise ships represent a unique environment for microbial transmission, few studies exist on this environment.
2. Integration of human and environmental samples to study the possible transmission of the pathogen and ARGs
3. Identification of ARGs which add a useful health dimension

Methodological weaknesses

1. Culturing bias: It is well known that most environmental microbes are not culturable under standard laboratory conditions. By only sequencing what grew on agar, the study is blind to the unculturable bacteria.
2. No diversity or community analysis: Alpha/beta diversity, ordinations PCoA and ecological distance metrics (Bray–Curtis, Jaccard). The statement that "surface and hand microbiomes were similar is not statistically supported.
3. No negative controls were mentioned in method section
4. For ARG data the CARD results are reported as present/absent which is not informative without normalization to total reads.

3. Results and discussion
The writing is generally clear and detailed, but too descriptive, the Results section reads like a table annotation rather than an analytical narrative.
Many large tables could be summarized graphically for example stacked bar plots of phyla/genera, heatmaps of shared taxa.
Correlation between surfaces and hands > Claimed strong correlation is not statistically shown.
The authors correctly note the limitations, for example the complete absence of fungi and the negligible detection of viruses (<0.01%). They attribute this to the DNA extraction kit, but the culturing step is the far more likely cause. Most environmental fungi and virus would not grow on these media in this timeframe.
Minimal discussion of environmental variables (humidity, cleaning agents, seawater aerosol, ventilation).
Fair Assessment
Despite these major limitations, the study is still useful but need a lot of work. This is not a comprehensive microbiome survey, but rather a great large-scale culturomics study focused on viable, clinically relevant bacteria.
From the following perspective the findings could be impactful:
It confirms that these *living* bacteria carry a significant arsenal of ARGs, highlighting a clear mechanism for the spread of antibiotic resistance.
It demonstrates that a wide variety of viable, culturable pathogens are present on surfaces and hands.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiome, environmental AMR, and climate-microbiology researcher

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 03 Dec 2025

Jörn Klein

We would like to thank the Reviewers for their thorough evaluation of our manuscript and for the constructive comments provided. We appreciate the opportunity to clarify several methodological aspects and to explain the rationale behind our analytical choices.
Both reviewers correctly noted that the culturing step weakens the scientific aims of the study, as most environmental microorganisms cannot be cultured under standard laboratory conditions. When planning the study, we also intended to rely solely on direct samples, as these would provide more reliable data and reflect the true microbial composition of the sampled surfaces. The cultures grown from fingerprint and surface samples on agar plates were collected only as a safety backup plan. Unfortunately, once we returned from the ship and processed the samples in the laboratory, it became clear that the direct samples did not contain sufficient DNA for Nanopore sequencing. For this reason, we decided to analyze the cultured samples instead.

Reviewer 1 suggested that the complete absence of fungi and the minimal detection of viruses can be better explained by the culturing step, rather than by the DNA extraction methods. We partly agree. It is true that viruses and many fungi cannot grow on the agar plates we used. However, human cells also cannot grow there, yet all our surface samples still contained human DNA. This indicates that non-culturable material - such as dead human cells - was still collected and included in the samples, although certainly in lower quantities than bacteria that were able to multiply. Moreover, some fungi (e.g., *Malassezia*) grow well on TSA, and in our previous experiments we frequently detected them in both hand and surface samples. In this study, however, *Malassezia* was absent from all samples, which is why we believe that the extraction method played a major role in these omissions.

As outlined above, the final sample composition was biased by both the extraction method and the culturing step. In addition, different culture media were used in different settings - such as MacConkey agar, which is designed to inhibit the growth of many species - introducing further variability. Both reviewers also pointed out the absence of diversity or community analyses (e.g., alpha/beta diversity or ecological distance metrics). We chose not to perform these analyses because, as described, our sample composition was biased. The culturing process shifted the microbial community, and the extraction method likely failed to capture certain organisms. Under these circumstances, we do not see a clear added value in applying diversity analyses to such a biased dataset. Instead, we tried to drawing conclusions that can be supported by the data available. For this reason, we emphasized the species that were detected in all samples. We believe this is a meaningful and more reliable approach, as it is less influenced by the culturing bias.

Reviewer 1 also highlighted that our results only confirm that these living bacteria carry a significant arsenal of ARGs. We do not fully agree. It is true that living bacteria - if compatible with the agar media - had the opportunity to multiply, increasing their relative abundance in the cultured samples. However, as demonstrated by the presence of human DNA, harvesting from the contact plates also captured non-viable material. This means that dead bacteria were sequenced as well, and their ARGs were detected, but at a lower frequency. The same reviewer suggested that the CARD ARG results should be normalized to read counts. We believe this is unnecessary. We described how the ARG detection data were biased by the culturing step, where viable bacteria outgrew the rest. Within these methodological constraints, we focused on what conclusions could reasonably be drawn: numerous antimicrobial resistance genes were found in an environment where healthy individuals spend their leisure time. We consider this observation potentially valuable from an infection control perspective, even though additional, better-controlled experiments are certainly required. The lack of positive and negative controls is a valid criticism. Unfortunately, these controls cannot be generated retrospectively; time passed, passengers have returned home, and the ship has since been sold.

Reviewer 1 further mentioned the minimal discussion of environmental variables such as humidity, cleaning agents, seawater aerosol, and ventilation. Cleaning agents and ventilation systems did not change during the sampling period. Humidity was not measured, but all samples were collected indoors on a closed ship environment operating at sea, where both temperature and humidity remained relatively stable. The reviewer also noted that we did not provide statistical evidence for a strong correlation between the microbiomes of hands and surfaces. We believe that Figure 9 offers an initial indication, as the same genera were present across sample types. However, conducting statistical analyses on a dataset that is clearly biased by culturing and extraction limitations would not be appropriate, and we chose not to present potentially misleading results.

Reviewer 1 is correct that our study is more a descriptive microbiological survey than a full-scale microbiome analysis. Despite the major limitations, we still believe the study provides useful insights. While strong conclusions cannot be drawn, the findings suggest that a wide variety of viable, culturable pathogens are present on surfaces and hands of healthy people, and that antimicrobial resistance genes can be detected even in such a low-risk setting. These observations are relevant from an infection control perspective and contribute an

additional viewpoint to current knowledge on the spread of AMR bacteria.

We fully acknowledge that this is only a first step in exploring this topic. Our project has finished, but we hope that other researchers will continue to investigate these questions with improved and more appropriate methodologies. The dissemination of our findings is only possible if the study is published.
Reviewer1 is right, our study is more a descriptive microbiological survey than a true microbiome study. We think that despite the major limitations, the study is still useful. Strong conclusions cannot be drawn, but it suggests that a wide variety of viable, culturable pathogens are present on surfaces and hands, and antimicrobial gens can be found in a such low-risk setting. We think these conclusions are important in infection control view, and can add a different view to our knowledge about the spread of AMR bacteria. We know that our study is just a first step to address this topic. Our project ended, but we would like to encourage other researcher to further address this issue – with better addressed methodology. But spreading the word is only possible is our study will get published, otherwise our result will be forgotten and other researchers had to start the work from the beginning.

Competing Interests: No competing interests were disclosed.