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Metagenome Analysis Reveals a Response of the Antibiotic Resistome to Mars-like Extraterrestrial Conditions

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Abstract

The spread of antibiotic resistance is becoming a serious global health concern. Numerous studies have been done to investigate the dynamics of antibiotic resistance genes (ARGs) in both indoor and outdoor environments. Nonetheless, few studies are available about the dynamics of the antibiotic resistome (total content of ARGs in the microbial cultures or communities) under stress in outer space environments. In this study, we aimed to experimentally investigate the dynamics of ARGs and metal resistance genes (MRGs) in Kombucha Mutualistic Community (KMC) samples exposed to Mars-like conditions simulated during the BIOMEX experiment outside the International Space Station with analysis of the metagenomics data previously produced. Thus, we compared them with those of the respective non-exposed KMC samples. The antibiotic resistome responded to the Mars-like conditions by enriching its diversity with ARGs after exposure, which were not found in non-exposed samples (*i.e., tet* and *van* genes against tetracycline and vancomycin, respectively). Furthermore, ARGs and MRGs were correlated; therefore, their co-selection could be assumed as a mechanism for maintaining antibiotic resistance in Mars-like environments. Overall, these results highlight the high plasticity of the antibiotic resistome in response to extraterrestrial conditions and in the absence of anthropogenic stresses. Key Words: Antimicrobial resistance—Extraterrestrial environment—Metal resistance—Kombucha multimicrobial community. Astrobiology 22, 1072–1080.

1. Introduction

Antimicrobial resistance has been spreading widely due to the massive use and misuse of antibiotics (Levy and Marshall, 2004); thus, this is becoming a major health concern worldwide (Rolain *et al.,* 2012). Antibiotic resistance genes (ARGs) have an environmental and ancient origin (D'Costa *et al.,* 2011; Perry *et al.,* 2016), given that they are part of the intrinsic or pre-resistome (*i.e.,* antibiotic resistance traits belong to metabolic pathways, having a role in the physiology of the bacterial cells), and they can rapidly evolve and become true ARGs, which is of clinical interest due to the increasing use and environmental release of antibiotics (Galán *et al.,* 2013). Therefore, it is pivotal to investigate the cycle of antibiotic resistome (total content of ARGs) and its dynamics with exposure to different

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environmental stresses to estimate the possible risk for humans, animals, and environmental health following the *One-health* (or *Ecohealth*) approach (Rabinowitz *et al.,* 2013).

Currently, an increasing number of studies are performed to improve our understanding of the dynamics and stress response of the antibiotic resistome in microbial communities in both indoor and outdoor environments. Indeed, in outdoor environments, the antibiotic resistome dynamics have been studied in aquatic ecosystems and in the air under different anthropogenic pressure levels (Corno *et al.,* 2019; Zhu *et al.,* 2021). Similarly, in indoor environments where there is a strict contact between people and where regular and rigorous cleaning procedures are applied (determining a high anthropogenic influence), the antibiotic resistome has been extensively investigated (Fahimipour *et al.,* 2018; Mahnert *et al.,* 2019; Ben Maamar *et al.,* 2020).

Of particular interest for NASA is the characterization of the microbiome that inhabits the indoor human-made environment of the International Space Station (ISS), and to ensure the health of an onboard crew (Mora *et al.,* 2016). Different studies have been performed, with a focus on antibiotic resistance, to define the antibiotic resistome from interior surfaces of the ISS (Urbaniak *et al.,* 2018) and investigate an increase and persistence of ARGs in real-time space missions (Singh *et al.,* 2018). To date, however, no studies have been proposed to investigate the antibiotic resistome outside the ISS, where anthropogenic pressure is absent and space environmental conditions impose stress for bacteria (Moissl-Eichinger *et al.,* 2016). There is, of course, no atmosphere outside the ISS, but inside one of the BIOlogy and Mars EXperiment (BIOMEX) compartments on the exposure platform EXPOSE-R2, a Mars-like environment was created. The atmosphere inside the BIOMEX is characterized as follows: CO_2 (95.5%); N_2, Ar, and O_2 (2.7%, 1.6%, and 0.15%, respectively): $\sim$370 ppm H_2O and a pressure of 980 Pa with a high level of radiation that reaches a total UV >200 nm fluencies of about 4.92×102 kJ m^{-2} for the unprotected sample (de Vera *et al.,* 2019). Furthermore, no studies to date have addressed the mechanisms involved in the spread of antibiotic resistance such as, for instance, metal resistance genes (MRGs) as co-selectors of ARGs in the absence of antibiotic selective pressure (Baker-Austin *et al.,* 2006), in extraterrestrial environments. Thus, no information is available about how ARGs and MRGs interact and evolve in the complete absence of anthropogenic pollution and in the outer reaches of a Mars-like environment.

In this study, we analyzed the shotgun metagenomics data (Góes-Neto *et al.,* 2021), previously experimentally produced, of the microbiomes of the Kombucha Mutualistic Community (producing known fermented product; KMC) samples, which were exposed and non-exposed to Mars-like environmental conditions (Podolich *et al.,* 2019). We aimed to (i) unveil the antibiotic and the metal resistome (total content of MRGs) of KMC samples exposed to Mars-like conditions; (ii) compare the composition of both resistomes (antibiotic and metal resistance genes content) between samples exposed to Mars-like environment and those non-exposed with the intent to evaluate differences in richness and relative abundance of ARGs and MRGs; and (iii) investigate whether MRGs could contribute to the maintenance and spread of ARGs by co-selection mechanism.

2. Materials and Methods

2.1. Experimental setup

Kombucha Mutualistic Community samples were used to investigate the influence of exposure to spaceflight and Mars-like conditions on antibiotic and metal resistomes by experimental settings previously described (Podolich *et al.,* 2019). Briefly, desiccated KMC pellicles of IMBG-1 ecotype (from the collection of IMBG, Kyiv, Ukraine) (embedded in the anorthosite/egg white mixture) were exposed for 18 months to simulated Mars-like conditions on low Earth orbit in a three-layer carrier mounted on the EXPOSE-R2 facility (Rabbow *et al.,* 2017) outside the ISS. The upper-level sample was unprotected from UV radiation (sample named KMC_1b), while samples from the middle and bottom levels were maintained in the darkness (samples named KMC_2b and KMC_3b, respectively). The non-exposed KMC sample was maintained in the laboratory at room temperature in the darkness (sample named KMC_4b). The samples exposed to Mars-like conditions and the non-exposed samples were reactivated, and aliquots of each were cultured monthly for 2.5 years (exposed cultured samples named KMC_1c, KMC_2c, and KMC_3c, non-exposed cultured sample named KMC_4c) as previously described in the work of Góes-Neto *et al.* (2021). Aliquots of all the samples and an aliquot of the initial KMC ecotype used for experiment preparation (named KMC_5) were processed for DNA extraction and sequencing (shotgun metagenomics) as already described by Góes-Neto *et al.* (2021).

2.2. Bioinformatic analyses

The community composition in target resistance inducing genes (resistome) was inferred by MetaStorm, an online platform for fully automated sequence analysis and visualization (Arango-Argoty *et al.,* 2016). Data and metadata analysis were retrieved from the Sequence Read Archive (NCBI, BioProject access numbers: PRJNA636820, PRJNA636837, PRJNA636891, PRJNA637016, and PRJNA637018).

The compressed raw read files were uploaded to MetaStorm and processed through a read matching pipeline. The read matching pipeline performed a quality filtering of reads, followed by annotation against one or more sequence databases selected by users. On average, more than 98% of raw reads passed the quality filtering step. Raw reads and high-quality reads are summarized in Supplementary Table S1.

For the functional annotation, functions were associated with the quality-filtered reads. Thus, lists of resistance genes that most probably belong to the query sequences were generated. MRG-like sequences were produced out of the annotation to the BacMet database of experimentally confirmed resistance genes (BacMet v2.0) (Pal *et al.,* 2014). ARG-like reads were obtained through the annotation to the DeepARG database (deepARG-DB-v1.1.1) (Arango-Argoty *et al.,* 2018). Alignment thresholds on sequence identity and minimum alignment length were set according to the work of Li *et al.* (2015) (E-value <1e-10, identity >90%, and minimum alignment length of 25aa). Copies of 16S rRNA genes were inferred by mapping against the 2013 release of the GREENGENES database (DeSantis *et al.,* 2006). The number of 16S rRNA gene-like hits per sample was used for the

normalization of ARG-like fragments and MRG-like fragments abundances, as described by Li *et al.* (2015). The processed data can be found by browsing the project name ''Kombucha mutualistic community resistome'' on the MetaStorm website (http://bench.cs.vt.edu/MetaStorm/). Pivot tables of relative abundance were created separately for the ARG-like sequences and the MRG-like sequences and, subsequently, exported for statistical analyses.

2.3. Statistical analysis

The statistical analyses were performed in the R environment (version 3.6.0) (R Core Team, 2019), considering nine samples: KMC_1b, KMC_1c, KMC_2b, KMC_2c, KMC_3b, KMC_3c, KMC_4b, KMC_4c, KMC_5. For the tests, we used as explanatory variables both the exposure to the Mars-like conditions, two groups: ''Exposed'' $n=6$ (KMC_1b, KMC_1c, KMC_2b, KMC_2c, KMC_3b, KMC_3c) versus ''Non-exposed'' $n=3$ (KMC_4b, KMC_4c, KMC_5), and the culturing, two groups: ''Cultured'' $n=4$ (KMC_1c, KMC_2c, KMC_3c, KMC_4c) versus ''Non-cultured'' $n=5$ (KMC_1b, KMC_2b, KMC_3b, KMC_4b, KMC_5).

Starting from ARGs and MRGs datasets obtained from shotgun metagenomics data (Góes-Neto *et al.,* 2021), we calculated the richness in ARGs and MRGs (as the number of different genes) of each sample and investigated the factor (exposure/culturing) that influences its variation through a negative-binomial linear model (NBLM). Similarly, the beta diversity, as abundance-based Bray-Curtis dissimilarity index, was determined and analyzed by using PERMANOVA. The differences in the total relative abundances of ARGs and MRGs were evaluated by ANOVA. In this case, the total relative abundances of the genes were used as response variables. Abundances were transformed, prior to analysis, into the arcsine of the square root of their value, due to the proportional nature of the data. The correlation between the total relative abundance of ARGs and MRGs was assessed by using Spearman rank correlation (considering them as correlated for rho >0.75). For the analysis, the MRGs dataset used was adapted by subtracting the genes also present in the ARGs dataset to avoid redundancy.

2.4. Network analysis

The co-occurrence between ARGs and MRGs was further investigated by network analysis, using the abundances retrieved by the KMC samples previously described (Góes-Neto *et al.,* 2021). A matrix of correlation was constructed through the Spearman rank correlation function of the *psych* package version 1.9.12.31 (Revelle, 2015). On this basis, a table was prepared that contained only those couples of genes strongly and significantly correlated (rho >0.8 and $p<0.01$). Then, the table was imported in Gephi (software v. 0.9.2) for network visualization (Bastian *et al.,* 2009).

3. Results

3.1. Antibiotic resistome composition

Antibiotic resistance genes were detected in all analyzed samples. In detail, a total of 200 different genes that belong to 22 resistance classes were found, with multidrug resistance as the most represented class in all samples (Fig. 1a). Richness of ARGs was significantly higher in the exposed samples than it was in the non-exposed samples (NBLM: $p=0.0174$) (Fig. 2a). Among 200 ARGs, 101 genes were exclusively present in the exposed samples. Four genes characterized the non-exposed samples, and 95 were shared between them (Table 1; for details, see Supplementary Table S2). Conversely, the culturing did not affect richness (NBLM: $p=0.7992$). Regarding the ARG composition, only 17.6 and 12.2% of the beta diversity were explained by the exposure and the culturing, respectively (Supplementary Table S3). The Principal Coordinates Analysis (PCoA) ordination, based on the Bray-Curtis dissimilarity index, did not show clear clusters, both in terms of exposure and culturing (apart for the KMC_2b sample, in a separated area of the figure) (Supplementary Fig. S1a). The relative abundance of ARGs ranged from 1.0×10^{-5} to 1.23×10^{-1} gene copies/16S rRNA gene copy in the exposed samples and from 1.0×10^{-5} to 4.83×10^{-2} gene copies/16S rRNA gene copy in the non-exposed samples. The most abundant ARG was *sul*1 (1.23×10^{-1} gene copies/16S rRNA gene copy, KMC_1b), followed by *mex*A (7.23×10^{-2} gene copies/16S rRNA gene copy, KMC_2b) and *mdt*B (5.39×10^{-2} gene copies/16S rRNA gene copy, KMC_1b) (Supplementary Fig. S2a). The total relative abundance was not significantly different among samples when considering the exposure to Mars-like conditions and the culturing (ANOVA: $p>0.05$), even if a quasi-significant increase in ARG abundances in the exposed samples was determined (ANOVA: $p=0.0578$) (Fig. 3a).

Among the 22 resistance classes, 17 occurred in exposed and non-exposed samples, while five classes were absent in the non-exposed samples; the *tet* (encoding tetracycline resistance) and the *van* genes (encoding for vancomycin resistance) were absent in non-exposed samples (Supplementary Table S4). Multidrug resistance genes were the most abundant class (1.95×10^{-1} copies/16S rRNA gene copy, KMC_2b). Sulfonamide resistance genes were the second most abundant class, with 1.23×10^{-1} copies/16S rRNA gene copy (KMC_1b), followed by aminocoumarin resistance genes (1.03×10^{-1} copies/16S rRNA gene copy, KMC_3b).

3.2. Metal resistome composition

Metal resistance genes were detected in all analyzed samples. A total of 179 different genes that belong to 17 resistance classes were found, and the multi-metal resistance was the most represented class in the different samples (Fig. 1b). The exposure to Mars-like conditions had only limited effect on the richness of MRGs (NBLM: $p=0.0708$) (Fig. 2b). Among 179 MRGs, 90 genes were characteristic of the exposed samples, three were only present in the non-exposed ones, and 86 were distributed in both (Table 1, Supplementary Table S5). The culturing had no effect on the richness (NBLM: $p=0.7371$). Conversely, taking the composition of MRGs into account, the culturing was the main factor (25.7%) that shaped the beta diversity (vs. 12.3% of the exposure) (Supplementary Table S3). Also, the PCoA ordination depicted samples that were partially separated on the basis of culturing as a factor (apart for the KMC_2b sample, in a distinct area of the figure) (Supplementary Fig. S1b). The relative abundance of MRGs comprised between 1.0×10^{-5} and 2.48×10^{-1} gene copies/16S rRNA

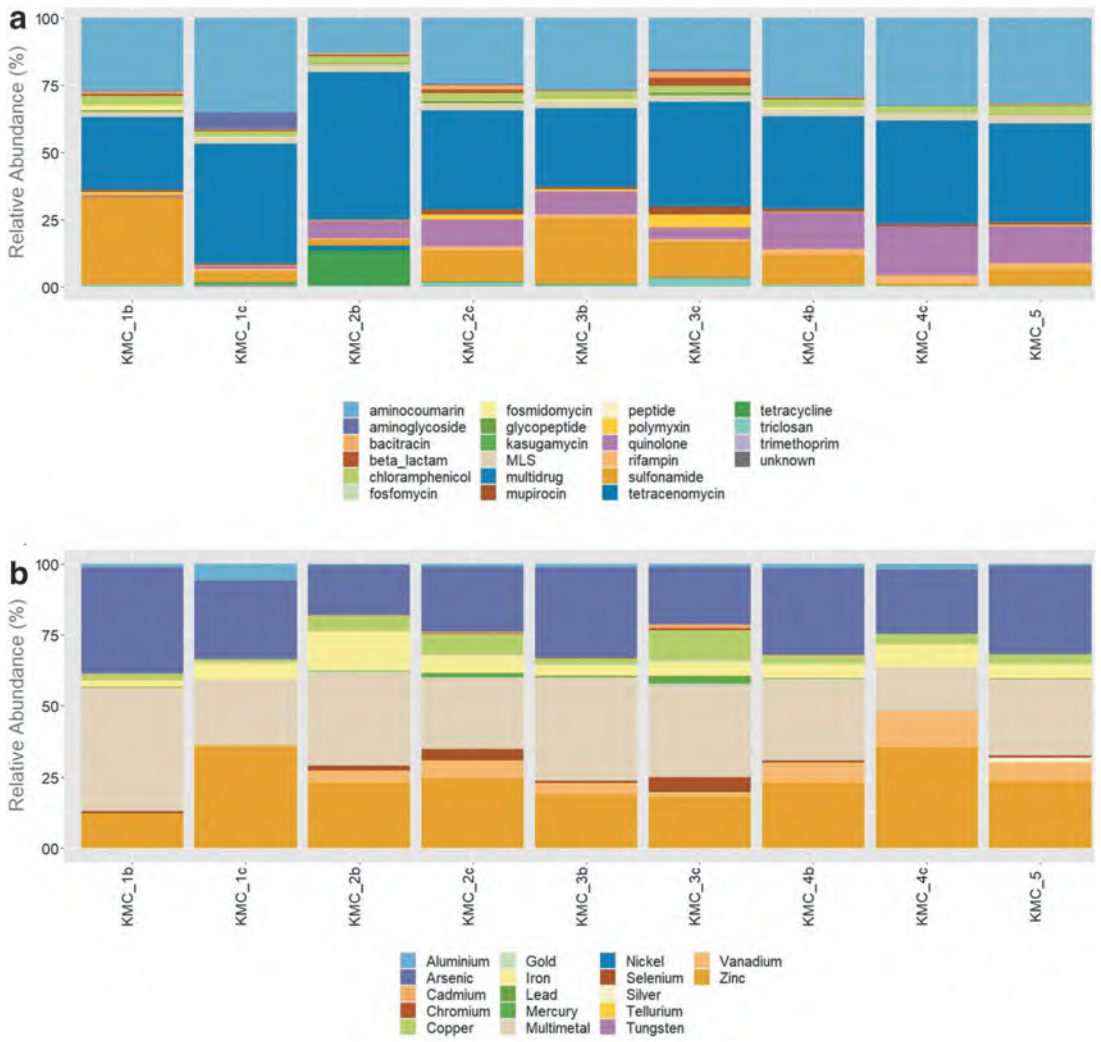

FIG. 1. Resistance class composition. Composition of the samples according to (**a**) antibiotic or (**b**) metal resistance classes. The relative abundances of specific resistance classes (against antibiotics or metals) were expressed as percentage for each sample.

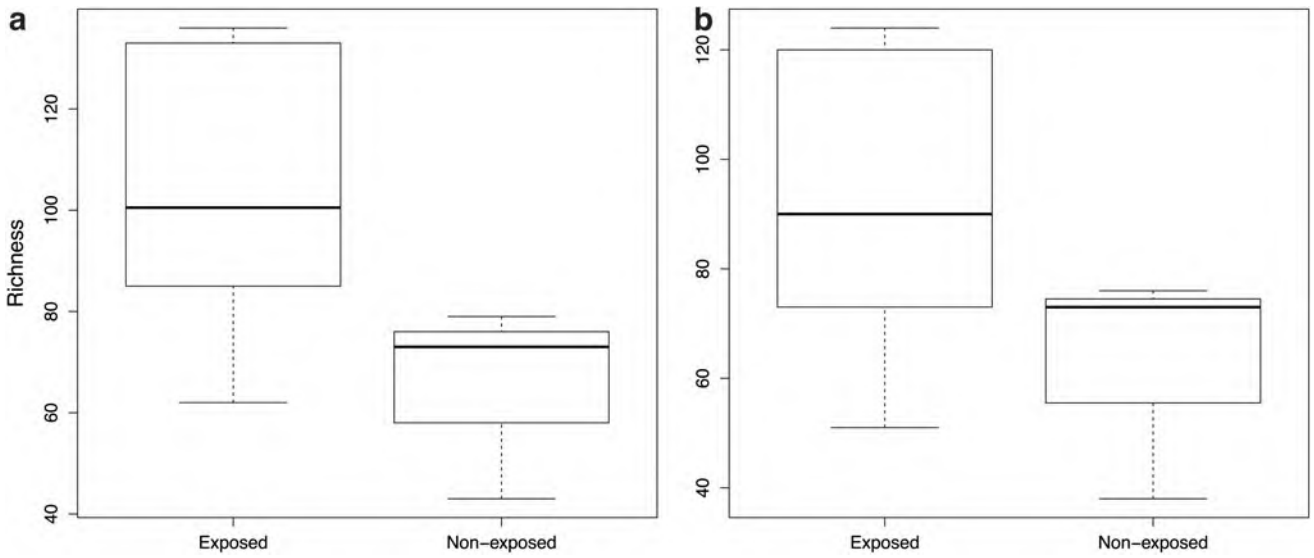

FIG. 2. Richness of resistance genes. Boxplot of the richness of (**a**) ARGs and (**b**) MRGs. The thick horizontal line represents the median, the box represents 50% of the values, the whiskers extend to the highest and lowest value within the 1.5 interquartile range.

Table 1. Number of Different ARGs and MRGs Present in the "Exposed" and "Non-exposed" Samples or Shared between Them

	Exclusive to			
	Exposed	*Non-exposed*	*Shared*	*Total*
ARGs	101	4	95	200
MRGs	90	3	86	179

gene copy in the exposed samples, and between 1.0×10^{-5} and 9.06×10^{-2} gene copies/16S rRNA gene copy in the non-exposed samples. The MRG with the highest abundance was *ars*H (2.48×10^{-1} gene copies/16S rRNA gene copy, KMC_1b), then *ars*C (2.37×10^{-1} gene copies/16S rRNA gene copy, KMC_1b), and *pst*B (1.07×10^{-1} gene copies/16S rRNA gene copy, KMC_1b). No patterns were observed with regard to the MRGs relative abundances (Supplementary Fig. S2b), and no significant differences in total relative abundances of MRGs were determined when considering both the exposure to Mars-like conditions and the culturing on Earth (ANOVA: $p > 0.05$) (Fig. 3b).

Among the 17 resistance classes, 16 were present in both exposed and non-exposed samples, while the genes that conferred resistance to tungsten and almost all the genes encoding for nickel resistance were absent in the non-exposed samples (Supplementary Table S6). Multi-metal resistance genes had the highest abundance (4.11×10^{-1} copies/16S rRNA gene copy, KMC_1b), followed by arsenic resistance genes (3.55×10^{-1} copies/16S rRNA gene copy, KMC_1b), and zinc resistance genes, as a third most abundant class with 1.40×10^{-1} copies/16S rRNA gene copy (KMC_1c).

3.3. Correlation between ARGs and MRGs

A positive correlation in the total relative abundances of ARGs and MRGs was obtained by Spearman rank correlation analysis (rho = 0.80, $p = 0.014$).

The co-occurrence of retrieved ARGs and MRGs was investigated by network analysis via Spearman rank correlation (Fig. 4), considering two elements as correlated for rho >0.8 and $p < 0.01$. The network was composed by 274 nodes (ARGs and MRGs) that formed 1447 interconnections (edges) and showed a modular structure (modularity index >0.4, as defined in Newman, 2006) that consisted of 46 modules (Fig. 4, Supplementary Table S7). Module A, shown in green, had the most complex correlations (involving 17.52% of network nodes), followed by modules B and C (each comprising 8.39% of nodes), shown in light blue and yellow, respectively (the other modules contained less than 6.5% of network nodes) (Fig. 4). In all the main modules, a co-occurrence between ARGs and MRGs was found. Module A was composed mainly by ARGs (54.2%), modules B and C mainly by MRGs (59.1% and 52.2%, respectively) (Fig. 4). Furthermore, module A comprised genes shared between Mars-like conditions exposed and non-exposed samples, whereas modules B and C contained only genes exclusive to the exposed ones (Fig. 4).

4. Discussion

Our findings, although limited to the analysis of the reads annotated as ARG-like sequences and, thus, based on a gene-centric approach (instead of genome-centric one), show that the antibiotic resistome in KMC samples responded to the extraterrestrial Mars-like conditions. Indeed, the total relative abundance of the detected ARGs was not significantly different between the exposed and non-exposed samples, with the former having a quasi-significant higher abundance than the latter. However, this result was affected by the high intragroup variability. This finding is not surprising, taking into account that, when bacteria are under stress conditions, they can respond by recruiting or activating ARGs or inducing physiological changes that impair or reduce their antimicrobial sensitivity (Poole, 2012).

With regard to the richness of the antibiotic resistome, it is surprising that, in the samples exposed to Mars-like

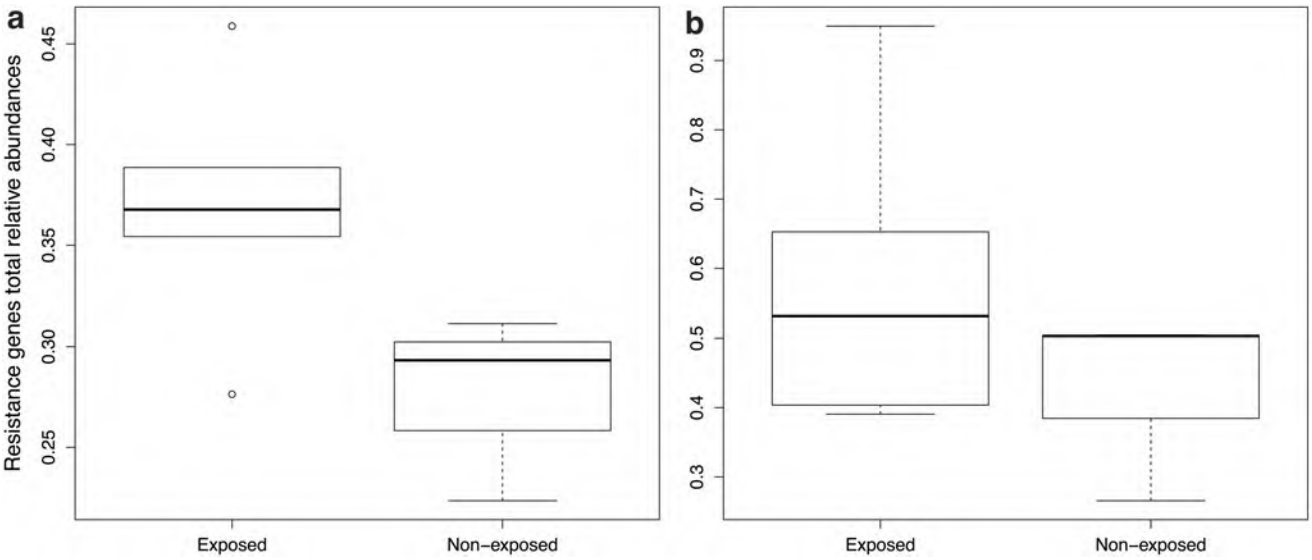

FIG. 3. Total relative abundances of resistance genes. Boxplot of the total relative abundances of (**a**) ARGs and (**b**) MRGs. The thick horizontal line represents the median, the box represents 50% of the values, the whiskers extend to the highest and lowest value within the 1.5 interquartile range.

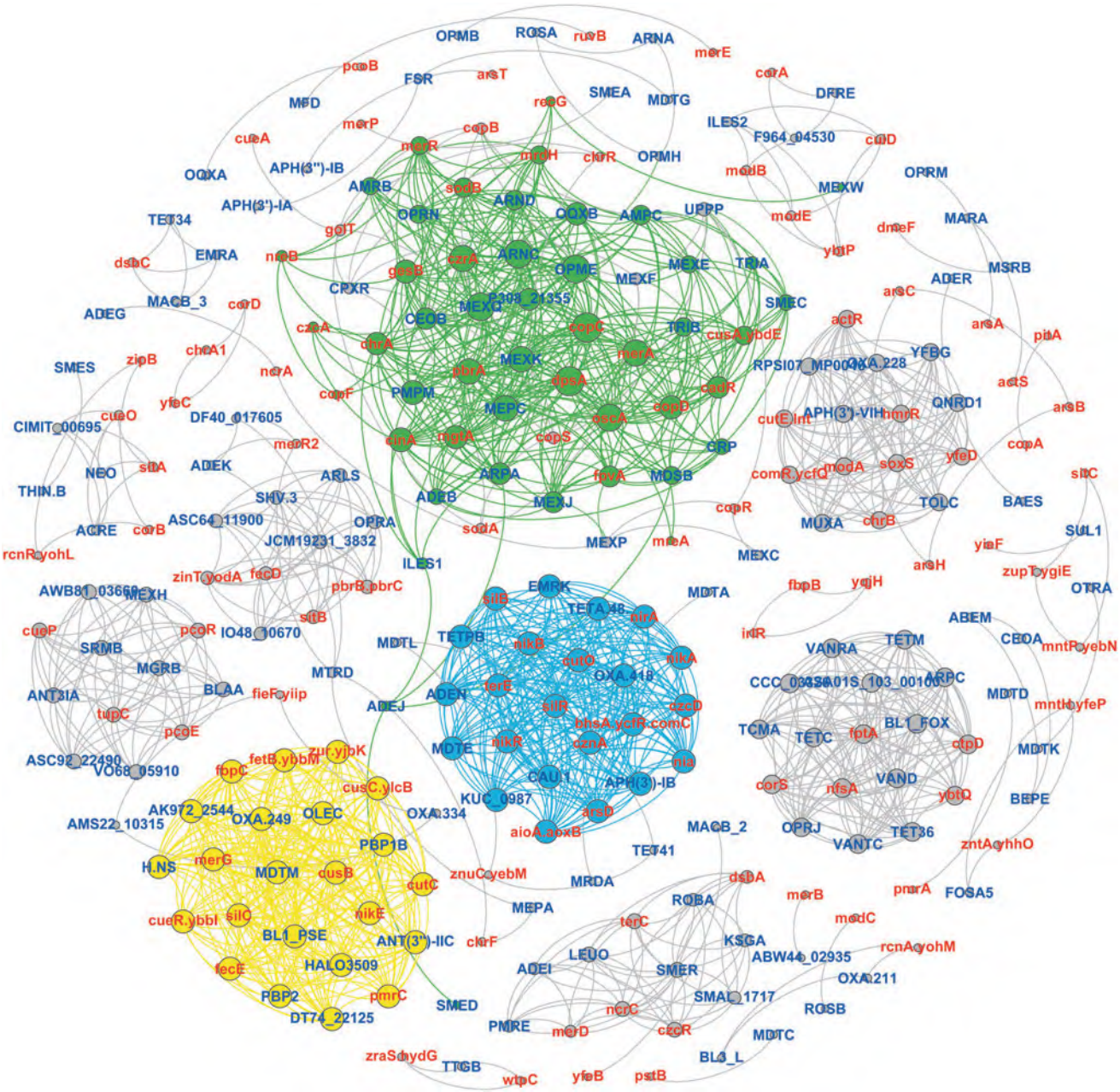

FIG. 4. Co-occurrence network. For network construction, elements with a significant and strong correlation (rho >0.8 and $p < 0.01$) were chosen. Node size is proportional to the number of edges. ARGs are indicated in blue, MRGs in red. Different colors were used for the modules in the network (Supplementary Table S7). In green: module A; in light blue: module B; in yellow: module C; in gray: modules with less than 6.5% of network nodes.

conditions, it was significantly higher in respect to that determined in the non-exposed samples. At the present, it is known that controlled indoor environments, such as that inside the ISS (defined as the most confined human-made inhabited environment to date and considered as an extreme environment) (Mora *et al.,* 2016; Checinska Sielaff *et al.,* 2019), are particularly selective for antibiotic-resistant bacteria (Mora *et al.,* 2016; Be *et al.,* 2017). Nonetheless, to the best of our knowledge, this is the first study in which the antibiotic resistome was analyzed outside the ISS without the contribution of the strict interaction between people.

Therefore, it is interesting to note that, in the absence of anthropogenic pressure, the antibiotic resistome in the samples exposed to Mars-like conditions evolved by increasing the number of different ARGs. In particular, it was only in these samples that different ARGs, that is, *aph(6)-IC*, *aph(3')-IB, mdtE, mdtL,* and *tet*M, all of which are classified at the highest risk for human health (Zhang *et al.,* 2021), were detected. Furthermore, the tetracycline (*tet*) and vancomycin (*van*) resistance genes were detected only in samples exposed to Mars-like conditions.

This indicates that the potential phenotype of resistance against tetracycline and vancomycin—the former belonging to the first generation of antibiotics and used in clinical practice to treat uncomplicated urogenital, respiratory, and other infections (Grossman, 2016) and the latter defined as a

''mainstay of antimicrobial therapy'' and considered a ''valuable clinical tool that provides effective gram-positive coverage at a low drug cost'' (Dilworth *et al.,* 2021)—appeared because of the exposure to Mars-like environmental stressors. Indeed, the factors influencing the recruitment of ARGs in the samples exposed to a Mars-like environment should be further investigated under these environmental conditions, that is, the composition of the atmosphere and Mars-like pressure.

The atmosphere in the present study was mainly composed of CO_2 that recently has proven to promote cell-to-cell contact and plasmid transfer, which determines the horizontal spread of ARGs (Liao *et al.,* 2019). Similarly, microgravity has previously been well documented as a condition that could induce the selection and spread of antibiotic resistance in both potential pathogenic (Tirumalai *et al.,* 2019) and non-pathogenic (Shao *et al.,* 2017) bacteria.

The increased richness of the antibiotic resistome in the samples exposed to Mars-like conditions was accompanied by an increased richness in the bacterial community—this was previously analyzed by Góes-Neto *et al.* (2021) and, thus, was not reanalyzed here—and this suggests that these increases were likely brought about by the antibiotic resistomes exposure to such conditions. This result is in contrast to what was observed by Mahnert *et al.* (2019) in microbial communities isolated inside the ISS. Indeed, Mahnert and colleagues (2019) found an increased antibiotic resistance in correspondence to a reduction of microbial diversity. This suggests that different mechanisms govern the ARGs dynamics inside and outside the ISS despite showing similar results.

The metal resistome was not significantly enriched in the samples exposed to Mars-like conditions with regard to total relative abundance or diversity. This finding clearly shows that the Mars-like environment did not affect the metal resistome composition. Nevertheless, with regard to the single MRG classes, except for aluminum, cadmium, selenium, and vanadium, there were MRGs detected for all the other classes in the Mars-like conditions exposed samples only, for example, the sole gene encoding for tungsten resistance and almost all the nickel resistance genes. Furthermore, MRGs, known as selectors of ARGs (Di Cesare *et al.,* 2016a) and extensively found correlated with ARGs in different environments (Di Cesare *et al.,* 2016b; Thomas *et al.,* 2020; Wang *et al.,* 2021), co-occurred with ARGs in both Mars-like conditions exposed and non-exposed samples.

It is noteworthy that the network analysis showed that two of three main modules were composed by genes exclusive to the samples exposed to Mars-like conditions. In particular, two ARGs, that is, *aph(3')-IB* and *mdtE,* classified at the highest risk for human health (Zhang *et al.,* 2021) and co-occurred with genes coding for different metal resistances, for example, arsenic, copper, nickel. This result enforces the hypothesis that MRGs could be involved in the co-selection of ARGs also in the extraterrestrial environment.

5. Conclusions

Our study overall, though it was carried out by using a gene-centric approach (annotating the reads as ARG-like sequences), revealed for the first time the dynamics of ARGs and MRGs in KMC samples exposed to a Mars-like environment and will help deepen our understanding of the behavior of the total resistome (total content of ARGs and MRGs) of microbial communities exposed to a unique harsh environment such as extraterrestrial space outside the ISS and its BIOMEX setup. Our results show that, in the absence of anthropogenic pressure as well, the antibiotic resistome responds to Mars-like conditions by enriching itself with undetected ARGs in respect to non-exposed ground-based samples and with potential new antibiotic resistance phenotypes (tetracycline and vancomycin resistance). Furthermore, MRGs could also contribute to the spread of ARGs in the extraterrestrial environment by a co-selection mechanism. Overall, these results contribute to a new scenario whereby ARGs can be selected and spread in microbiomes exposed to harsh extraterrestrial conditions, also in the absence of anthropogenic pressure. This outcome calls for follow-up experiments focused on analysis of the dynamics of ARGs with the intent to contribute to a deeper understanding of the mechanisms involved in their selection and spread (*e.g.,* co-selection, horizontal gene transfer) in Mars-like environments and the threat posed by antibiotic resistance in future spaceflights.

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Author Contributions

Raffaella Sabatino: conceptualization, statistical analysis, writing, review, and editing. Tomasa Sbaffi: bioinformatic analysis, writing, review, and editing. Gianluca Corno: conceptualization, writing, review, and editing. Daniel Santana de Carvalho: bioinformatic analysis, writing, review, and editing. Ana Paula Trovatti Uetanabaro: writing, review, and editing. Aristóteles Góes-Neto: writing, review, and editing supervision. Olga Podolich: writing, review, and editing. Natalia Kozyrovska: writing, review, and editing. Jean-Pierre de Vera: writing, review, and editing, leading and scientifically coordinating the BIOMEX project. Vasco Azevedo: writing, review, and editing. Debmalya Barh: writing, review, and editing. Andrea Di Cesare: conceptualization, writing, supervision, review, and editing.

Conflict of Interest Statement

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper.

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Supplementary Material

Supplementary Table S1
Supplementary Table S2

Supplementary Table S3
Supplementary Table S4
Supplementary Table S5
Supplementary Table S6
Supplementary Table S7
Supplementary Figure S1
Supplementary Figure S2

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Abbreviations Used

ARGs = antibiotic resistance genes
BIOMEX = BIOlogy and Mars EXperiment
ISS = International Space Station
KMC = Kombucha Mutualistic Community
MRGs = metal resistance genes
NBLM = negative-binomial linear model
PCoA = Principal Coordinates Analysis