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Microbial community, pathogenic bacteria and high-risk anti-biotic resistance genes at two tropical coastal beaches adjacent to villages in Hainan, China

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Abstract

Objective. The aim of the study was to explore the correlation between characteristics of microbial community, pathogenic bacteria and high-risk antibiotic-resistant genes, between coastal beaches and a multi-warm-blooded host, as well as to determine potential species biomarkers for faecal source contamination on tropical coastal beaches in China.

Methods. The 'One-Health' approach was used in a microbiological study of beaches and warm-blooded hosts. The microbial.community was analyzed using 16S rRNA gene amplicons and shotgun metagenomics on Illumina NovaSeq.

Results. The Chao, Simpson, Shannon, and ACE indices of non-salt beach were greater than those of salt beaches at the genus and OTU levels ($P < 0.001$). Bacteroidota, Halanaerobiaeota, Cyanobacteria, and Firmicutes were abundant on salt beaches ($P<0.01$). Human-sourced microorganisms were more abundant on salt beaches, which accounted for 0.57%. *Faecalibacterium prausnitzii* and *Eubacterium hallii* were considered as reliable indicators for the contamination of human faeces. High-risk carbapenem-resistant *Klebsiella pneumoniae* and the genotypes KPC-14 and KPC-24 were observed on salt beaches. Tet(X3)/tet(X4) genes and four types of MCR genes co-occurred on beaches and humans; MCR9.1 accounted for the majority. Tet(X4) found among Cyanobacteria. Although rarely reported at Chinese beaches, pathogens, such as *Vibrio vulnificus*, *Legionella pneumophila*, and *Helicobacter pylori*, were observed.

Conclusions. The low microbial community diversity, however, did not indicate a reduced risk. The transfer of high-risk ARGs to extreme coastal environments should be given sufficient attention.

Key words

microbial community; carbapenem-resistant *Klebsiella pneumoniae*; antimicrobial resistance; antibiotic-resistant genes; coastal beach; one health

INTRODUCTION

Coastal beaches are exposed to specific environmental stresses and have a variety of microecological characteristics [1], being reservoirs of microorganisms and antibiotic-resistant genes (ARGs) [2, 3]. Contamination of wastewater from humans and livestock, coastal aquaculture, water or air circulation, has a great impact on the beach microbial community [3, 4]. The coastal ecology could be more suited for halophiles than the open sea [1]. At present, studies have explored and analyzed marine microorganisms, but the source of microbial pollution and high-risk ARGs has been relatively ignored, particularly at tropical coastal salt beaches [4].

In general, polluted beaches can be evaluated for various faecal-sourced microbial indicators, such as *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus* from warm-blooded animals [5–7]. Anti-microbial resistance (AMR) and ARGs in different marine areas have certain commonalities, for example, sulfonamide, β lactamase, and integrase ARGs, which are widely distributed [8, 9], and regional specificity. Few studies have reported that AMR mediates resistance to colistin and tigecycline in the marine environment, and the two antibiotics are the last-line defence of antibiotic-resistant Gram-negative bacteria. Carbapenem-resistant *Enterobacter* has been reported in wastewater, soil, and health population, some of which carried colistin-resistant mobile MCR genes [10–13]. New studies have reported that the tigecycline-resistant gene tet(X) and its variants found in shrimp, ducks and livestock [14, 15], faeces, soil, and sewage [16]; however, but none of them have been reported in coastal beach environments.

The two types of beaches included in this study were located in western China's southernmost tropical island province. One of the salt beaches has a history spanning more than 900 years. More human leisure activities and some domestic pollution were observed at the salt beach and its extension area. The other beach did not have salt leaching, and all the samples were a mixture of mud and sand.

The two types of beaches are close to the villages. It was hypothesized that the microbial community, high-risk ARGs, and pathogenic bacteria at the two beaches were directly

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related to human and warm-blooded animal faeces. Thus, the study was designed from the perspective of 'One Health', and samples of the faeces of humans, pigs, cattle, and sand were collected and analyzed.

'One Health' is an effective method for controlling the microorganism risk across regions and species. In 2022, the first plan for the 'One Health Joint Plan of Action' was launched by FAO, UNEP, WHO, and WOAH (WHO, 2022) [17]. However, implementing the plan remains difficult in many local regions.

This study focuses on the microbial communities of salt beaches and non-salt beaches by analyzing 16S rRNA gene amplicons and metagenomic sequencing, pathogenic bacteria, and high-risk ARGs that mediate colistin, tigecycline, and carbapenem resistance. The host bacteria for important ARGs and their distribution were also analyzed. The pollution of beach microorganisms was traced to the potential host of cattle, pigs, and village residents, and the multifactor correlation characteristics of the two types of microbial community are discussed.

MATERIALS AND METHODS

Hainan Island is the southernmost island province in China, where in some regions the environmental hygiene is poor. The two beaches are both within 50 m of village residences, and almost no livestock is found in the villages around the salt beach, while free-range cattle and captive pigs are found in the villages near the non-salt beach. Two types of coastal beaches in Danzhou County, Hainan Province, namely, a salt beach SAB (including salt pans and a 50 m extended area) and a non-salt beach (NSB), were selected. The centre of SAB is located at longitude 109. 233 and latitude 19. 855, and the NSB is located at longitude and latitude 109. 221 and 19. 835, respectively. The number of samples in SAB and NSB was 15 and 14, respectively. The distance between the stations in SAB and NSB was 4,400 m. The sample sites were partitioned on the basis of the spacing at the two beaches. All beach samples were used for sequencing of 16S rRNA gene amplicons, among which seven were used for metagenomic sequencing.

All human faecal samples were collected using a convenient method in the vicinity of the salt beach from male and female residents over 18 years of age who lived in the villages within the last 6 months. All of them gave an informed consent to the collection of faecal samples, and the study was approved by the Ethics of Committee of Hainan Medical University. A total of 32 qualified faecal samples from human, 21 from cattle, and 25 from pigs were collected, all of which were sequenced for 16S rRNA gene amplicons, and 10 faecal samples from humans were further studied by metagenomic sequencing.

The environmental samples were collected on 13–14 July 2021. Beach sediment samples were collected within 5 cm by using the five-point method and mixed for each sample. The samples were placed at –20 °C and transported in dry ice to the Meji Biotechnology Company within 48 h for amplicon 16S rRNA gene and metagenomic sequencing.

16S amplicon sequencing, metagenomic sequencing, and diversity analysis. DNA extraction, PCR amplification, Illumina MiSeq sequencing for 16S rRNA amplicons, library construction, sequence quality control, genome assembly, and metagenomic sequencing were performed, in accordance with the reference, with some modifications (Supplementary S1) [18]. The V3–V4 region of the 16S rRNA gene was amplified by PCR with the primers 338F (5′-ACTCCTACGGGAGGCAGCAGCAG-3′) and 806R (5′-GGACTACHVGGG TWTCTAAT-3′). α diversity was analyzed using the Shannon, Simpson, Chao, and ACE indices. β diversity analysis among the microbial communities was determined by partial least squares discrimination analysis (PLS-DA). ANOSIM analysis was used to identify the division rationality of groups. PERMANOVA and SIMPER were used to calculate the average dissimilarities in the bacterial community structures among samples, and to ascertain which categories was responsible for the observed differences. The linear discriminant method of LefSe species differences (LDA) was used to analyze the microbial communities in different groups.

Traceability analysis for faeces. The relative abundance of OTUs was analyzed on the two beaches using the Megi Bioplatform to trace the source of faecal pollution. The abundance information of matched sequences among different groups of species was calculated. The data were analyzed using the online tool of the Majorbio Cloud Platform (https://cloud.majorbio.com/ page/ tools/) [19]. Common species and abundance between the beach and host were analyzed to explore the source of pollution.

Analysis of pathogens and microbial indicators. Community analysis of bacteria included common environmental microorganisms, faecal indicators, and clinically-relevant pathogens. The abundance distribution of pathogenic bacteria was obtained by collecting genes at the family and genus levels, and annotating species in detail through the species and function annotation module. Correlation analysis was performed by using the Rank sum correlation test in spss16.0.

Functional annotation of CARD resistance genes. Antibiotic resistance annotation was conducted using Diamond and the CARD database (CARD v3.0.9) with an e-value cutoff of $1e^{-5}$. Species and function cluster analysis was performed on high-risk mobile carbapenem-resistant genes, the tigecycline-resistant gene tet(X), and the colistin-resistant gene family (MCR). Species and function clustering were performed on the Megi Biotechnology Company platform. Characteristic distribution in samples and the species of host bacteria were taken into consideration.

Statistical analysis. The Wilcoxon rank sum test was used to analyze the inter-group differences in α diversity. β diversity analysis of PLS-DA was based on the non-parametric factorial KW sum-rank test. ANOSIM, PERMANOVA and SIMPER analysis was used according to the dissimilarity method by Bray and Curtis. Linear discriminant analysis (LDA) was also performed with an LDA score of >3.5, $P < 0.05$.

RESULTS

The samples from the two beaches were sequenced for 16S rRNA amplicons. A total of 61 phyla and 1230 genera were

identified in the SAB samples, whereas 66 phyla and 1431 genera were identified in the NSB samples.

By metagenomic analysis, pathogenic microorganisms, including *Helicobacter pylori*, *Vibrio cholerae*, *Vibrio vulnificus*, *Legionella pneumophila*, *Campylobacter*, *Pseudomonas aeruginosa*, and *Salmonella*, were primarily distributed. Notably, carbapenems-resistant *Klebsiella pneumoniae*, which is considered by the WHO to be clinically relevant and extremely dangerous [20], was found at the extended salt pan of SAB.

The data can be found on NCBI, the 16S amplicon sequencing ID: SRP467857, PRJNA1030958, which can be accessed via: https://www.ncbi.nlm.nih.gov/sra/PRJNA1030958. For the metagenome analysis data, the ID is SRP468049, and the Accession for SRA data – PRJNA1030995, which can be accessed via: https://www.ncbi.nlm.nih.gov/sra/PRJNA1030995.

Microbial diversity and species distribution at two beaches was assessed based on 16S rRNA gene amplicon sequencing. *The α* diversity of Shannon, Simpson, ACE, and Chao indices of SAB was less than that of NSB at the genus and OTU levels (*P < 0.05*. Supplementary2 A, B, C, D), and the difference was statistically significant *(P<0.001,* Wilcoxon signed-rank test).

PLS-DA analysis of beta diversity was investigated, with the result that the difference between the two beaches was statistically significant at the genus level. The four samples from the extension area of the salt pan were separated from the other SAB samples at the genus level (Fig. 1A). The phylum distribution is shown in Figure 1B.

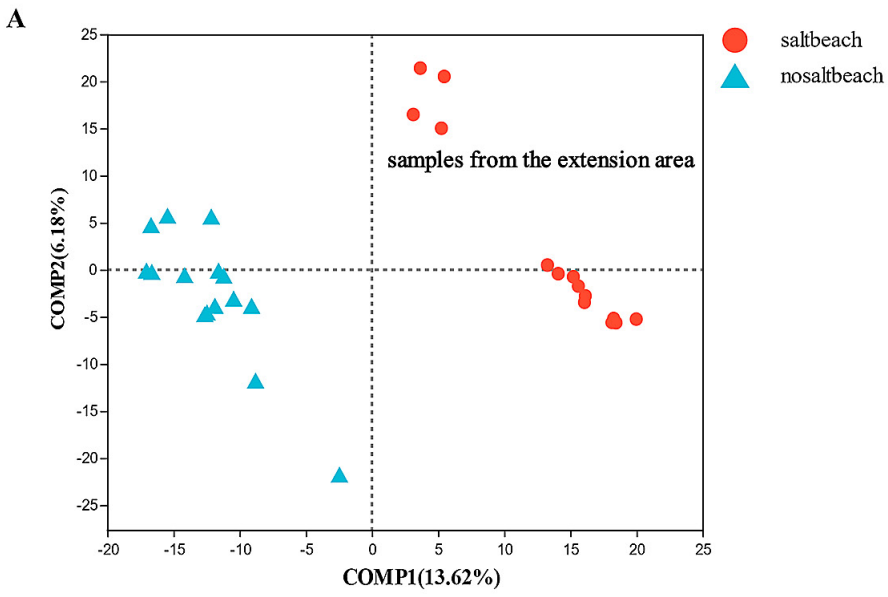
Results of ANOSIM showed that the groups division between SAB and NSB was statistically significant (R=0.4508, P=0.001<0.05), as well as within the SAB group (R=0.8971, P=0.002<0.05). The division of groups showed statistical significance. The distance box plot on the genus and OTU levels between SAB and NSB is shown (Supplementary 3 A, B).

The difference at the phylum level was further analyzed by LEfSe (LDA>3.5). Four phyla showed high abundance at SAB, and five phyla showed high abundance at NSB (Fig. 2A). In addition, Halanaerobiaeota (a kind of halophilic bacterium), accounted for the vast majority at the phylum level, including 98.5% at SAB and 1.50% at NSB.

Considering that most of the abundant genera were not identified, the difference in species at the family level was analyzed (P<0.05, Wilcoxon rank sum test) (Fig. 2B).

PERMANOVA and SIMPER analysis for 16S rRNA gene amplicon sequencing. Comparison of bacterial communities among the groups of two beaches was performed. PERMANOVA showed that the difference in abundance among the predefined groups of samples, as well as within SAB, was statistically significant (P<0.01). SIMPER analysis was also performed for the top 100 abundant genera, with the result that the dominant contribution of the known genus at the two beaches was *Woeseia, Bacillus, Robiginitalea, Halanaerobium*, and *Vibrio*. Among the 20 genera, four were halophilic bacteria, namely, *Halanaerobium*, *Idiomarina*, *Aliifodinibius*, and *Salinibacter*, which contributed 3.55%, 2.77%, 2.51%, and 2.33% of the overall difference, respectively.

Source tracking of microorganisms and exploring new faecal indicators for the beaches. Source tracking was explored using the data at the OTU level by the function module on the Meggie Biotech platform by 16S rRNA gene amplicon sequencing [19] (Ren et al., 2022). The abundance



B

Beaches / Species	Proteobacteria	Bacteroidota	Gemmatimonadota	Actinobacteriota	Firmicutes	Desulf bacterota	Acidobacteriota	Chloroflexi
Saltbeach	24.88%	18.87%	10.88%	7.86%	7.80%	4.81%	3.48%	4.51%
Non-saltbeach	28.95%	12.07%	3.86%	9.84%	2.58%	8.02%	8.56%	7.84%

Figure 1 β diversity differences in PLS-DA and phylum composition between the salt beach and non-salt beach

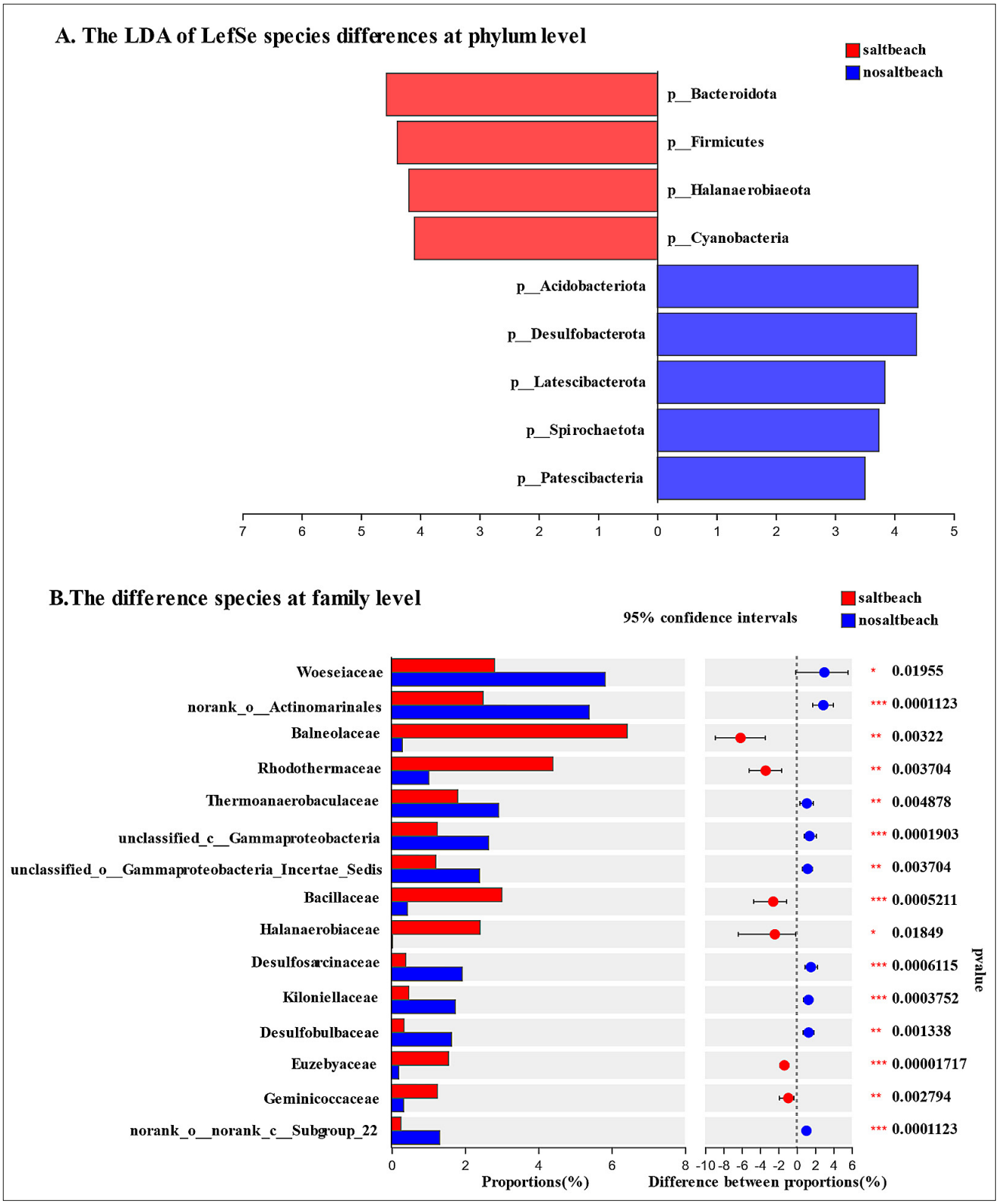


Figure 2. Comparison of differences by using linear discriminant analysis (LDA) of LEfSe at the phylum level between the two beaches

of matched sequences among the different groups of species was calculated by using the module. The contribution of contamination from humans, pigs, and cattle accounted for 0.57%, 0.12%, and 0.09%, respectively. For NSB, the contribution of contamination from humans, pigs, and cattle was 0.09%, 0.16%, and 0.18%, respectively (Supplementary 4A).

Further analysis showed that the common genera between SAB or NSB beaches and humans accounted for 34 and 30, respectively, whereas at the species level, their common species accounted for 92 and 62, respectively. The intersection of SAB and cattle was 32, and that of NSB and cattle was 89 at the genus level; the number of common species was 66 and 175. The following unique common genera between the host

Table 1 Abundance and distribution of microbes in beaches and hosts

Categories		Salt beach	Non-salt beach	Abundances of hosts and ratio			
				Humans	Cattle	Pigs	Ratio* of bacteria abundance between 2 groups of hosts
Species level	*Faecalibacterium prausnitzii*	26	2	2962	370	11	>8.00
	Eubacterium hallii	7	0.1	2373	0.1	5	>474.60
	Escherichia coli (usual indicator)	23	3	2164	223	80	>9.70
Genus level	*Blautia*	28	3	4179	129	72	>32.65
	Eubacterium hallii group	7	0.1	2532	55	30	>346.03
	Faecalibactrium	26	3	3152	382	12	>8.25
	Enterobacter	6	2	1196	1	0.1	>1196
	UCG-005	2	17	99	9285	910	>10.20
	Lactobacillus	32	11	20	590	3280	>5.56

*Ratio: high abundance divided by low abundance

and two beaches are shown in Supplementary 4B: human and salt beach (12) > cattle and salt beach (11) > pig and salt beach (2); cattle and non-salt beach (42) > pig and non-salt beach (12) > human and non-salt beach.

The abundance of the top 50 species (Supplementary 5A) in two beaches and feces were explored to determine potential faecal indicators in a beach environmen,t by comparing the abundance ratio among humans, cattle, and pigs. Five rarely reported genera in faeces of human, cattle and pigs, as well as two species in humans, were found for their specific and dominant abundance in one host (Tab. 1). The abundance among the indicator species is shown in Supplementary 5A and 5B. Thus, with regard to abundance and species characteristic, the results were consistent with source tracing on the platform.

Composition and distribution of pathogens based on metagenomes. A total of 21,592 and 21,836 species were observed at SAB and NSB, respectively, while 1,247 and 817 unique common species were found between humans and SAB or NSB, respectively. In addition, the total ARGs found at beaches accounted for 780.

Thirteen important pathogen-related genera both at beach and human feces are shown in Figure 3. High-risk species were also observed, including *Vibrio vulnificus, Pseudomonas aeruginosa, Haemophilus influenzae, Legionella pneumophila, Streptococcus pneumoniae, Staphylococcus aureus, Campylobacter jejuni,* and *Shigella sonnei*.

The correlation of pathogens between the abundance logarithm of the salt beach and human faeces was statistically significant (r=0.522; *P*=0.020), whereas the difference was not significant between the non-salt beach and human faeces (r=0.360; *P*=0.142).

Characterization and association analysis of antibiotic-resistant genomes based on metagenomics. Of the important antibiotic-resistant bacteria, carbapenem antibiotic-resistant *Enterobacter* (CRE) was found at SAB and in human feces. The main genes observed in CRE included NDM-6, NDM-8, KPC-14, and KPC-24; another important ultrabroad spectrum β-lactamase-producing *Enterobacter* (ESBLs) showed TEM, SHM, CTX-M, and OXA genes.

Remarkably, carbapenem-resistant *Klebsiella pneumoniae* is considered as the primary clinical risk of antibiotic-resistant bacteria [20]. The carbapenem-resistant genes KPC-14 and KPC-24, as well as the tigecycline-resistant gene tet(X4), were found in SAB and human faecal samples, whereas the

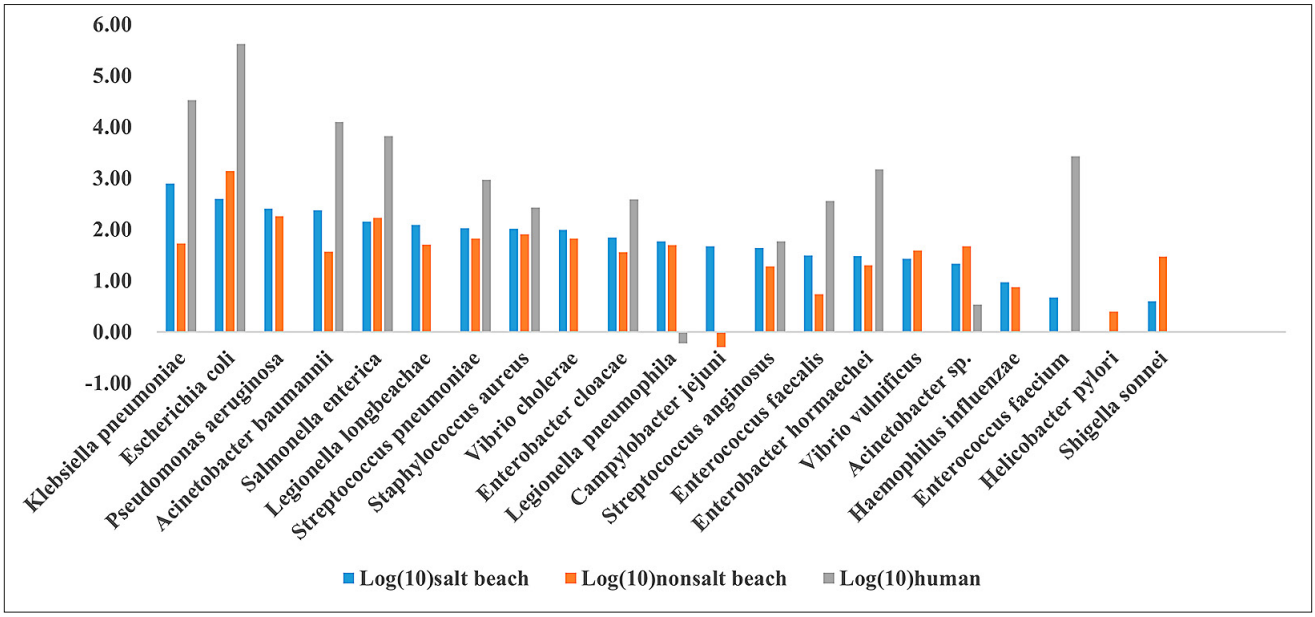


Figure 3. Relative abundance of clinically-relevant pathogens found at beaches and in humans (transformed abundance of log10

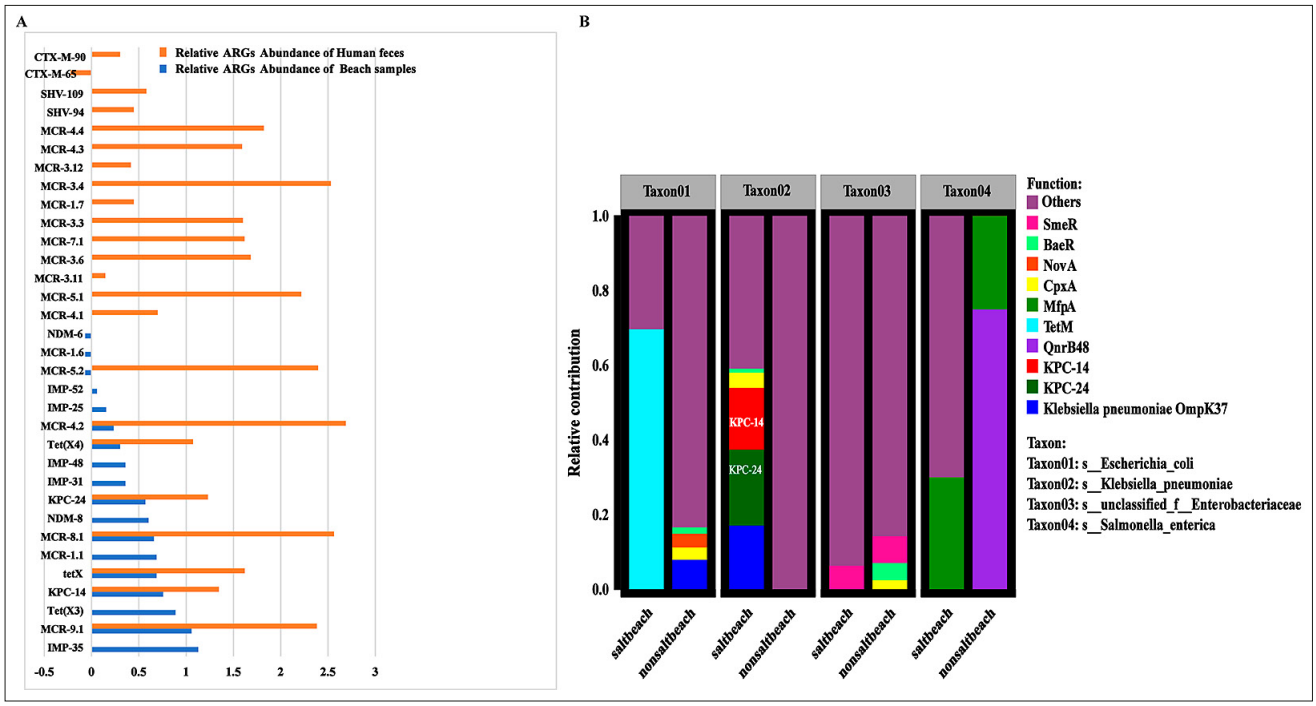


Figure 4 (A) Relative abundance of high-risk antibiotic-resistant genes at beaches. (B) Distribution of antibiotic-resistant genes and hosts of *Enterobacteriaceae* at two beaches

colistin-resistant genes MCR4.2 and MCR8.1 were found in human faecal and NSB samples. MCR5.2 and MCR9.1 were observed in samples obtained from the two beaches and human feces. In addition, the MCR family of colistin-resistant genes was widely found on beaches and in human feces, with 17 MCR family members, and only 11 genes were observed in human feces (Figure4A). Among the main types of MCR genes, MCR9.1 had the highest abundance. Furthermore, MCR1.1 was only observed on beaches and not in human feces. The relative abundance and distribution of important ARGs are shown in Figure 4A.

In general, Enterobacteriaceae is an indicator of warm-blooded host faecal pollution, which is a common risk to humans. The ARGs found in Enterobacteriaceae were further analyzed, and the distribution of ARGs at the family Enterobacteriaceae is shown in Figure 4B.

Among *Enterobacteriaceae*, *Klebsiella pneumoniae* carrying KPC24 accounted for 20.22%, and KPC-14 accounted for 16.63%. In addition, *Salmonella enterica* carrying qnrB48 accounted for 74.99% at NSB (Fig. 4B). The genes NDM-6, NDM-8, and IMP can cause resistance to carbapenems, and these gene were observed on beaches but not in human faeces. β ultrabroad spectrum lactamase of CTX-M-65 was found only on beaches, whereas the gene was reported by a study of faeces conducted by bathing beach practitioners at the southern end of Hainan [21].

The high-risk genes were further constructed to analyze the source sample and host strains (Tab. 2). The host of MCR genes was found in Acidobacteria, Proteobacteria, Chloroflexi, and tet(X3)/tet(X4) in four species of bacteria. Proteobacteria was the most dominant phylum and was involved in the hosting of high-risk ARGs. In this study, cyanobacteria carrying tet(X4) was a widely distributed group of bacteria, which can grow in an extreme environment. In addition, extremely high abundances and varieties of tetracycline-resistant genes were found at the beaches.

Table 2. Samples and host strains for high-risk resistant genes

CARD ARO	ARO name	Sample	Length	Phylum	Species
ARO:3004684	MCR-9.1	non-salt beach	384	Chloroflexi	*Anaerliae_bacterium_CG2_30_64_16*
ARO:3004684	MCR-9.1	non-salt beach	672	Acidobacteria	*Acidobacteria bacterium Mor1*
ARO:3004684	MCR-9.1	non-salt beach	513	Proteobacteria	*Deltaproteobacteria bacterium*
ARO:3004684	MCR-9.1	non-salt beach	681	Acidobacteria	*Acidobacteria bacterium*
ARO:3004684	MCR-9.1	salt beach	504	Acidobacteria	*Acidobacteria bacterium*
ARO:3004684	MCR-9.1	salt beach	504	Acidobacteria	*Acidobacteria bacterium*
ARO:3004684	MCR-9.1	salt beach	504	Acidobacteria	*Acidobacteria bacterium*
ARO:3002356	NDM-6	nonsalt beach	255	Proteobacteria	*Sphingomonadaceae_bacterium*
ARO:3002358	NDM-8	nonsalt beach	702	Proteobacteria	*Sphingomonadaceae_bacterium*
ARO:3004719	Tet(X3)	salt beach	414	Proteobacteria	*Candidatus_Competibacter denitrificans*
ARO:3004719	Tet(X3)	salt beach	612	Proteobacteria	*Inmirania thermothiophila*
ARO:3004719	Tet(X3)	salt beach	954	Chloroflexi	*Chloroflexi bacterium*
ARO:3004720	Tet(X4)	salt beach	321	Cyanobacteria	*Pleurocapsa minor*

DISCUSSION

α diversity was higher at NSB than at SAB, whereas the presence of pathogens and high-risk ARGs was not the same. Among the indices, Chao and ACE can reflect the rare species diversity of samples, whereas Shannon and Simpson can reflect the community abundance and evenness of sample. Proteobacteria dominated the samples at two beaches, as similarly reported in other marine environments [22]. SAB was contaminated with human faeces, particularly in the extended area. From the perspective of microbiology population, Bacteroidota, Halanaerobiaeota, and Cyanobacteria were among the key organisms with higher abundances at SAB. Bacteroidota are usually used as an indicator of warm-blooded animal faecal contamination in warm-blooded animals [23, 24]. The high salinity might be the main influencing factor for Halanaerobiaeota. The relative abundance of the genus *Salinibacter* at SAB was 290.43 times higher than at NSB, which is similar to reports that the bacterium was extremely halophilic from saltern crystallizer ponds [25].

By analyzing the characteristic species and their abundance, the result was similarly consistent in finding the main warm-blooded host of faecal contamination at beaches. The 16s rRNA gene amplicon sequencing combined with metagenomic sequencing is important for tracing the source of microbial species and ARG contamination, which is similar to previously reported studies [26]. As the abundance of *Eubacterium hallii* dominated in human faeces among the three hosts and the beaches, this could be a reliable indicator for human faeces contamination, although its role as an indicator of this species of bacterium has not been reported in the environmental setting.

Additionally, the 'One Health' approach can lead to novel faecal microorganism indicators in warm-blooded animals, and molecular markers can be designed on the basis of the indicators to trace the sources of the microorganisms. However, in the current study, only three kinds of hosts were collected because the poultry and wildlife in the two villages were limited.

With the exception of the hygienical indicators, *Vibrio cholerae*, *Legionella pneumophila*, *Legionella longbeachae*, and *Helicobacter pylori*, were first reported at the beaches in south China, and only a few other cases have been observed in other marine environments [27–29] which indicate a latent health risk for residents. *Legionella longbeachae* was not abundant but was observed at beaches and in human faeces. In cases reported in many regions, *Salmonella enterica*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Campylobacter jejuni* were the main pathogens in the contaminated environment [28, 29]. However, further studies on species characteristics could improve the validity of hygienic indicators. Similarly, ARGs can also be used as pollution source indicators of coastal water [25]. Notably, the types and abundance of clinically relevant pathogens were more at SAB than that at NSB, particularly *Vibrio cholerae*, which can cause class A infectious diseases: *Vibrio vulnificus*, which poses a serious risk of infection; *Legionella pneumophila* and *Legionella longbeachae*, which cause lung infection; and *Helicobacter pylori*, which increases the risk of stomach cancer.

As for the ARGs of MCR and tet(X) family, the MCR family has a higher abundance and more diverse types. Apart from MCR9.1 found in other reports, the newly-discovered MCR5.2 has been widely detected at beaches and in human faeces. At present, mobile MCR genes have been reported worldwide, and MCR4 was deduced to be sourced from the marine environment [30], and only MCR4.2 was observedin the current study. Acidobacteria was the main host of MCR9.1; tet(X4) was observed at both beaches and in human faeces, whereas tet(X3) was observed only at the beaches. Based on previous reports, the tet(X) family has been found in livestock, and many new studies have found tet(X4), tet(X3), tet(X5.2), and tet(X5.3) host Acinetobacter from birds, pigs, water bodies, and sewage environments [14, 15, 31, 32]. In addition, the positive rate of tet(X4) accounted for 25.3% locally in Hainan [16]. Thus, tet(X4) must be further studied with regard to its origin, intermediate habitat, genetic evolutionary pathway, and epidemic characteristics [16]. Similar to other studies, the quinolone-resistant genes were the dominant ARGs in coastal waters, and qnrB48 was abundant in the family of Enterobacteriaceae.

Many ARGs as MCR and tetX family genes are mobile genetic elements, which might be affected by multiple factors, such as high temperature, salinity, and strong UV radiation as are most ARGs [33, 34]. These elements are usually transmitted across species in animals, plants, humans, and the environment, and can also be transferred from non-pathogenic bacteria to pathogenic bacteria, or they can enter the human body via food, water, aerosols, and contact [34]. Therefore, attention should be paid to the prevention of potential risks of MCR, tet(X4), and KPC ARGs in tropical coastal environments.

CONCLUSIONS

ARGs, CRE, and major pathogens were found in coastal beaches adjacent to villages, which may be due to faecal contamination. From the perspective of 'One Health', combining 16S rRNA gene amplicon sequencing with metagenomics can effectively determine the microbial contamination indicators and trace the source. Low microbial community diversity in beaches with leisure functions does not indicate a reduced risk. Several molecular microorganism marker indicators can be developed on the basis of the microecological genomics in coastal environments. Thus, further research is urgently needed to trace the source of regional contamination, explore the evolution of the microbial community, and investigate the transmission characteristics of high-risk resistant ARGs at tropical coastal beaches.

Supplementary Materials.

S1 – experiment methods and bioinformation analysis for 16S rRNA amplicon sequencing and metagenomic sequencing.

S2 – differences in α diversity on four indices between the salt beach and non-salt beach (Fig. 1 A: Shannon index; B: Simpson index; C: Ace index; D: Chao index).

S3 – Figure A, B, C, and D. ANOSIM analysis of the distance box plot on the genus and OTU levels between SAB and NSB, as well as within the SAB group.

S4 – Figure A. Source tracking of faecal contamination of two marine beaches; B. Unique common genera between beaches and warm-blooded hosts.

S5 – Figure A. Community heat map analysis for beaches

and hosts at species level. B – community heat map analysis for beaches and hosts at genus level.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Hainan Medical University.

Informed Consent Statement. An informed consent was obtained from all subjects involved in the study.

Data Availability Statement. All relevant data are within the manuscript and its additional files.

Conflicts of Interest: The authors declare no conflict of interest. The providers of funds had no part in the design of the study, collection of data, analyses, interpretation of data, writing of the manuscript, or in the decision to publish the results.

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AppendixA
All the supplements can be accessed via: https://pan.baidu.com/s/1FlPI2KAKKBJR2aw7OnsOFQ?pwd=1111

S1. Experimental details and bioinformation analysis for 16S rRNA amplicon sequencing and metagenomic sequencing;

Figure S2. Differences in α diversity on four indices between the salt beach and nonsalt beach (Fig. 1 A – Shannon ndex; B – Simpson index; C – Ace index; D – Chao index).

Figure S3. A. Distance box plot on the genus level for SAB and NSB groups by ANOSIM analysis. B. Distance box plot on OTU level for SAB and NSB groups by ANOSIM analysis

Figure S4. A – Source tracking of faecal contamination of two marine beaches; B – Unique common genera between beaches and warm-blooded hosts.

Figure S5. A – Community heat map analysis for beaches and hosts at the species level. B – Community heatmap analysis for beaches and hosts at the genus level.

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