Critical Care

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Elevated antibiotic resistance gene abundance of ICU healthcare workers, a multicentre, cross-sectional study

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Abstract

Objective Studies suggest that the colonization of multidrug-resistant organism in the gut of healthcare workers is similar to that of healthy individuals. However, due to exposure to medical environments, is the abundance of antibiotic resistance genes (ARG) in the gut of ICU healthcare workers higher than that of healthy individuals?

Design Prospective, multicentre, cross-sectional study.

Setting Eight medical centers in China, recruiting from January 2024 to February 2024.

Participants 303 Healthy people (201 ICU healthcare workers and 103 healthy controls) were screened and 290 Healthy people (191 ICU healthcare workers and 99 healthy controls) were included in analysis.

Main outcome measures Fecal samples were collected and subjected to metagenomic sequencing. We compared the total ARG abundance, ARG diversity, and gut microbiome composition between the two groups.

Results After adjusting for age, sex, and body mass index, ICU healthcare workers exhibited a significantly higher total ARG abundance compared to healthy controls (fold change = 1.22, 95% CI: 1.12–1.34, $p < 0.001$). The β-diversity of ARG between the two groups differed significantly ($p = 0.001$). No significant linear or nonlinear relationship was observed between the duration of ICU occupational exposure and ARG abundance (p for overall = 0.96, p for nonlinear = 0.84).

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Conclusion In this prospective, multicenter study, we found that ICU healthcare workers exhibit significantly higher gut ARGs abundance compared to healthy controls. Meanwhile, ICU healthcare workers, including physicians, nurses, and nursing assistants, have a different composition of gut ARGs compared to healthy individuals.

Trial registration: NCT06228248.

Keywords Antibiotic resistance genes, Healthcare worker, ARG, ESKAPE

Introduction

Antimicrobial resistance (AMR) is a growing global health threat to humans, animals, and the environment, with deaths from AMR expected to rise in the coming decades. [1, 2] Medical institutions, due to frequent antibiotic use, are significant sources of antibiotic resistance genes (ARGs), with higher levels found in hospital wastewater and indoor air compared to outside environments and the use of antibiotics and disinfectants further promotes the spread of resistance [3–5]. Healthcare workers play a key role in the transmission of ARGs, with studies showing microbial transmission between healthcare workers and patients, especially in intensive care units (ICUs) [6–8].

Cross-contamination by medical staff is considered a major factor in the transmission of MDROs [9]. Although some studies suggest similar levels of multidrug-resistant organism (MDRO) colonization in healthcare workers who care for MDRO patients and those who do not, these findings are limited by bacterial culture methods and small sample sizes [10, 11]. Metagenomic approaches, including deep sequencing, provide a more accurate assessment of healthcare workers'role in ARG and MDRO transmission [12].

AMR is a global health concern, as resistance genes spread within and beyond healthcare settings [13]. The One Health framework, which connects human, animal, and environmental health, has yet to be fully explored within hospitals [14, 15]. We hypothesize that ICU healthcare workers have higher gut ARG abundance than healthy controls, with a correlation to occupational exposure duration. We aim to use deep metagenomic sequencing to assess ARG levels in ICU healthcare workers, contributing to the understanding of AMR dynamics in clinical settings.

Methods

Trial design and participants

This prospective, multicentre, cross-sectional study investigates the differences in the abundance of ARGs in the gut microbiome between ICU healthcare workers and the healthy controls. Conducted in eight medical centers across Zhejiang and Henan provinces, China, the study is registered on clinicaltrials.gov (NCT06228248, Registered on January 18, 2024). Inclusion criteria were 1). Age greater than 18 years old. 2). Fully understand and sign the informed consent form. Exclusion criteria include 1). Existence of gastrointestinal diseases, malignant tumors, and psychiatric disorders; 2). broad-spectrum antibiotic use within 6 months, defined as vancomycin, linezolid, piperacillin-tazobactam, imipenem-cilastatin, meropenem, cefepime, ceftazidime, and aztreonam, which are not available in outpatient settings. Fluoroquinolones were not included in this category; 3). Pregnant women; 4). The healthy controls were recruited from routine health check-ups and had no previous or current engagement in medical-related work.

Regarding the sample size estimation, given the lack of well-established baseline data on ARG abundance in healthy individuals, our calculation was based on a clinically meaningful effect size rather than a precise prior estimate. Assuming a 20% increase in ARG abundance in ICU healthcare workers compared to controls, with a 2:1 ratio of workers to controls, a significance level of 0.05, and 80% power, 168 ICU workers and 84 controls were required. After accounting for a 10% sample exclusion, the final target was 185 ICU workers and 93 controls.

Sample processing and sequencing data analysis

Fecal samples were collected, stored at −80 °C, and processed at Zhejiang University School of Medicine. Total fecal DNA was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN) following the manufacturer's instructions. DNA concentration and purity were measured using NanoDrop 2000 and TBS-380, and quality was assessed using 1% agarose gel electrophoresis. The DNA was fragmented to an average size of ~400 bp using Covaris M220, followed by library construction using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific). Paired-end sequencing (2 ×150 bp) was performed on the Illumina NovaSeq X Plus platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Raw sequencing data were processed using Fastp v0.23.2 to remove adapters and low-quality reads. Human-derived sequences were filtered using Bowtie2 v2.4.1 against the human reference genome (GRCh38). Taxonomic profiling was performed with Kraken2 v2.1.3,

using the Kraken_PlusPF database. ARG identification was conducted with RGI v6.0.3 against the CARD v3.2.9 database. ARG abundance was normalized as reads per kilobase per million mapped reads (RPKM), and ARG richness was quantified as the total number of identified ARGs. More detailed information can be found in the study protocol.

To determine the bacterial sources of ARGs, we matched sequencing read IDs between the outputs of Kraken2 and RGI. Specifically, we first identified ARG-containing reads using RGI and extracted their corresponding read IDs. These IDs were then cross-referenced with the taxonomic assignments from Kraken2, allowing us to trace each ARG-positive read back to its bacterial host. This approach enabled precise linking of ARGs to specific bacterial species, thereby facilitating host-level analyses of ARG distribution.

Statistical analysis

The primary outcome is to assess whether ICU healthcare workers have higher ARG abundance than healthy controls, with ARG abundance quantified as RPKM. Generalized linear models (GLMs) were used for all comparisons, including both unadjusted and adjusted analyses (accounting for gender, age, and BMI). RPKM values were log-transformed to approximate normality prior to GLM analysis, so the fold-change was derived as e^{β}, where β represents the estimated regression coefficient from the model.

The secondary outcome is to explore if ICU exposure duration correlates with ARG carriage. Restricted cubic splines (RCS) were used to model exposure duration. Subgroup analyses considered age, gender, BMI, and underlying diseases, with interaction terms included in the models. Sensitivity analyses were conducted by geographic location and professional roles.

On assess variations in the primary outcome across subgroups, we performed subgroup analyses based on demographic and clinical characteristics, including underlying diseases, age, gender, and BMI. Participants were categorized by underlying diseases, median age, gender, and BMI (< 18, 18–24, > 24). GLM was applied with log-transformed ARG abundance as the dependent variable, adjusting for gender, age, and BMI. Interaction terms were included to test if the relationship between healthcare worker status and ARG abundance differed across subgroups. Sensitivity analyses were also conducted based on geographic location and professional roles of ICU healthcare workers. More details could be found in supplementary materials.

All statistical tests and associated diagram generation were conducted in R software v.4.2.0. Alpha-diversity analysis, Principal Coordinates Analysis (PCoA), and differential abundance analysis were performed using the R package pctax (v.0.1.2, https://github.com/Asa12138/pctax). The rms package v.6.2.0 was used for constructing the regression models. A two-sided p-value of < 0.05 was considered statistically significant.

Results

Participants and baseline characteristics

A total of 201 ICU healthcare workers and 103 healthy controls met the inclusion criteria and underwent screening (Fig. 1). Three healthy controls and seven ICU healthcare workers were excluded due to the presence of gastrointestinal diseases. Stool samples were obtained and sequenced from 194 ICU healthcare workers and 100 healthy controls. One healthy control and three ICU healthcare workers were excluded because their stool samples did not meet the sequencing quality control standards. In the end, data from 191 ICU healthcare workers and 99 healthy controls were included in the analysis. The enrolled ICU healthcare workers were younger (33 vs. 42, $P < 0.001$) and had lower BMI (22.55 vs. 23.66, $P = 0.03$) than the healthy controls (Table 1). Besides, 75.92% of ICU healthcare workers were female, which is higher than the healthy controls (75.92% vs. 46.46%, $P < 0.001$). As for the medical profession, 13.09% of health workers were physicians, 72.77% were nurses, and 14.14% were nursing assistants. Regarding underlying diseases, the vast majority of participants had no underlying conditions

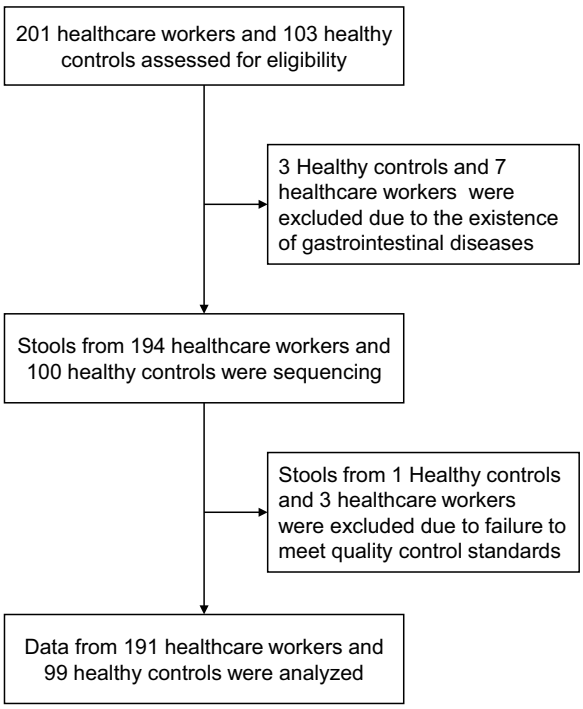

Fig. 1 Flow chart of the study

Table 1 Baseline characteristics of healthcare workers and health controls

Variables	Total (n = 290)	Healthcare Workers (n = 191)	Healthy Population (n = 99)	*P* value
Age, year, median (IQR)	36 (30, 45)	33 (29, 39)	42 (37, 51)	<.001
BMI, median (IQR)	23.04 (21.12, 25.34)	22.55 (20.82, 25.23)	23.66 (21.86, 25.56)	0.03
Female, n (%)	191 (65.86)	145 (75.92)	46 (46.46)	<.001
Medical profession, n (%)				–
Physician	25 (8.62)	25 (13.09)	–	
Nurse	139 (47.93)	139 (72.77)	–	
Nursing Assistant	27 (9.31)	27 (14.14)	–	
Underlying disease, n (%)				
Hypertension	11 (3.79)	4 (2.09)	7 (7.07)	0.08
Hashimoto's thyroiditis	3 (1.03)	3 (1.57)	0 (0.00)	0.52
Hyperthyroidism	2 (0.69)	1 (0.52)	1 (1.01)	1.00
Others	9 (3.10)	5 (2.62)	4 (4.04)	0.76
Location, n (%)				–
Zhejiang	134 (46.21)	134 (70.16)	/	
Henan	57 (19.66)	57 (29.84)	/	
Smoker, n (%)	26 (8.97)	8 (4.19)	18 (18.18)	<.001
Drinker, n (%)	38 (13.10)	22 (11.52)	16 (16.16)	0.27
Hospital Working Time, month, median (IQR)	–	98 (40, 143)	–	–
ICU Working Time, month, median (IQR)	–	68 (26, 120)	–	–

Descriptive statistics summarize the baseline characteristics of healthcare workers (n = 191) and health controls (n = 99). Continuous variables such as age, BMI, and working time are presented as medians with interquartile ranges (IQR). Categorical variables like gender, medical profession, underlying diseases, and lifestyle factors (smoking and drinking) are presented as frequencies and percentages. *P*-values indicate statistical significance based on Mann–Whitney U tests for continuous variables and chi-square or Fisher's exact tests for categorical variables

(91.38%). Among the healthy controls, 87.88% (87 participants) had no underlying condition.

The total ARG abundance in ICU healthcare workers was significantly higher than in the healthy controls

The average sequencing depth is 16.35 G with high-quality scores (Q20: 98.74%, Q30: 96.09%), and details were provided in Table S1. A total of 2,320 ARGs were identified through comparison with The Comprehensive Antibiotic Resistance Database (CARD). The GLM analysis revealed that the total relative abundance of ARGs in ICU healthcare workers was significantly higher than in the healthy controls ($p < 0.001$), with a fold change of 1.22 after adjusting for gender, age, and BMI (Fig. 2A and B), as these confounders were proven to have significant impacts on gut microbiome composition and ARG expression. To investigate the potential sources of ARGs in the gut microbiota, we analyzed ARGs associated with major bacterial pathogens, including the ESKAPE group (Fig. 2C). Compared to healthy controls, healthcare workers exhibited significantly higher ARG abundance derived from Enterococcus faecium, Klebsiella pneumoniae, and Enterobacter spp. including E. coli.

The composition of ARGs in ICU healthcare workers is more diverse

Subsequently, we assessed the α and β diversity of ARG in the two groups based on the pre-set research protocol. The richness ($p < 0.001$) and Shannon index ($p = 0.02$) of the ARGs of ICU healthcare workers was significantly greater compared to the healthy controls (Fig. 3A). The β-diversity between the two groups was also significantly different (Adonis $R^2 = 0.0396$, $p = 0.001$) (Fig. 3B). Additionally, ICU healthcare workers exhibited a substantial number of significantly upregulated ARGs in their gut microbiota (Fig. 3C). 243 ARGs were significantly upregulated (p-adj < 0.05, log2 fold change > 1) in ICU healthcare workers compared to 9 significantly downregulated ARGs. Among the 243 significantly upregulated ARGs, several genes exhibited substantial increases in relative abundance. The qnrE1 gene, which is associated with resistance to quinolone antibiotics, showed a log2 fold change of 14.03 (p-adj = 0.02). Additionally, several genes from the ACT and MIR families, which confer resistance to β-lactam antibiotics, were markedly upregulated, including ACT-12 (log2 fold change = 11.23, p-adj = 0.03) and MIR-12 (log2 fold change = 11.17, p-adj = 0.04). Comparison with the CARD identified that the elevated ARGs in ICU healthcare workers were primarily

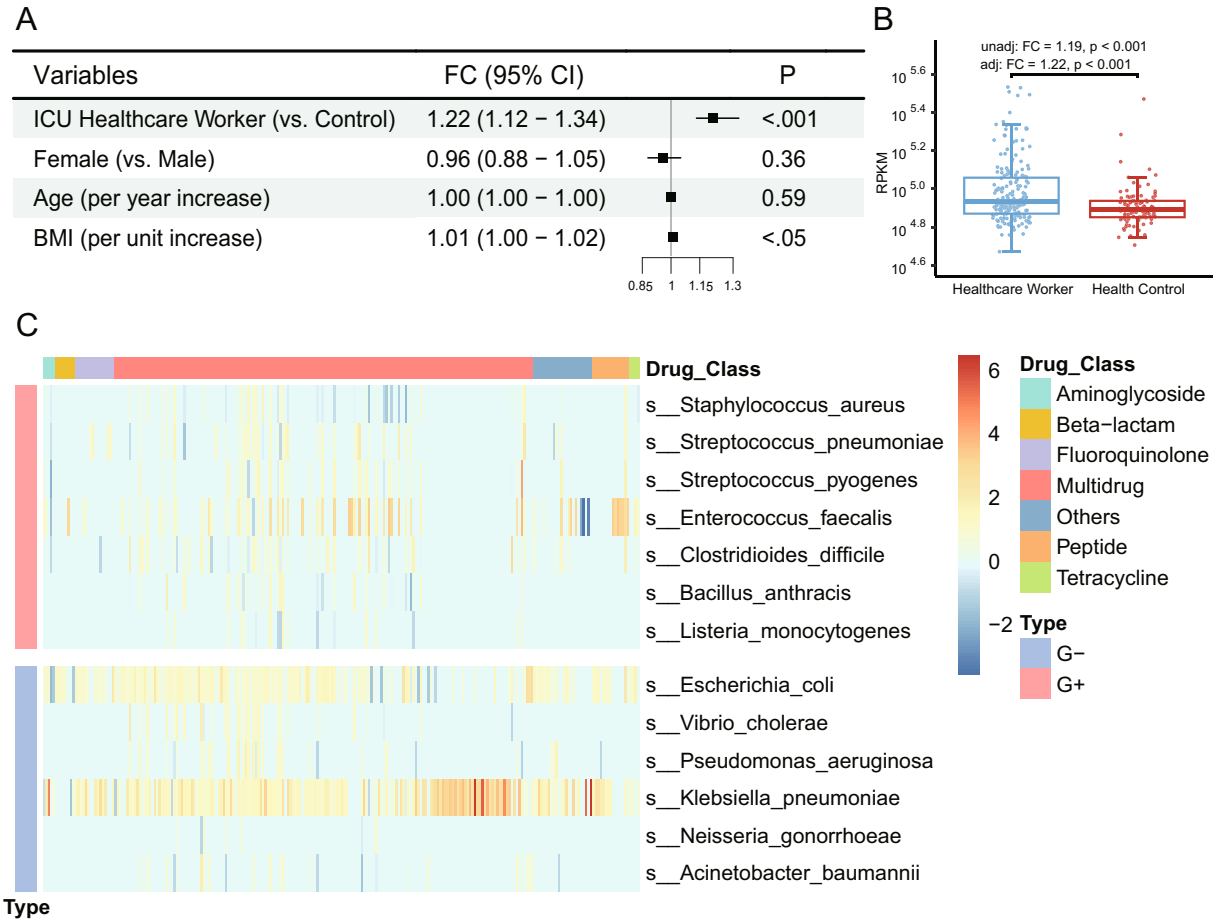

Fig. 2 Total ARG abundance in healthcare workers and health controls. **A** Forest plot presenting the results of the regression analysis, including adjusted fold-change (FC), 95% confidence intervals (CI), and *P*-values for key variables (ICU healthcare workers vs. control, gender, age, and BMI). **B** Boxplot illustrates the distribution of total ARG abundance in healthcare workers and healthy controls. The horizontal line within each box represents the median ARG abundance. The unadjusted fold change between the two groups is 1.19 (95% CI: 1.10–1.29, $p < 0.001$), while the adjusted fold change, controlling for age, gender, and BMI, is 1.22 (95% CI: 1.12–1.34, $p < 0.001$). **C**. Heatmap of log2 fold changes in ARG abundance (Healthcare Worker vs. Healthy Control) across representative gut bacterial species. Rows represent bacterial species, including both Gram-positive (G^+, upper panel) and Gram-negative (G^-, lower panel) bacteria, while columns represent individual ARGs grouped by antibiotic class. ARGs derived from Enterococcus faecium, Klebsiella pneumoniae, and Enterobacter spp. were markedly elevated in healthcare workers.

associated with fluoroquinolones, peptides, carbapenems, and cephalosporins resistances (Fig. 3D). We also found that the upregulated ARGs in ICU healthcare workers were mainly contributed by bacteria such as *f_Enterobacteriaceae, g_Bifidobacterium,* and *s_Collinsella_aerofaciens* (Fig. 3E).

ARG abundance was not significantly associated with the duration of ICU work

To further assess whether the duration of ICU occupational exposure is associated with increased ARG abundance, the multivariable linear regression model was used. The analysis demonstrated that there was no significant linear or nonlinear relationship between ICU working time and ARG abundance (p for overall = 0.96, *p* for nonlinear = 0.84), with the regression coefficient (β) approaching zero (Fig. 4).

Subgroup analysis reveals significant associations between higher age, overweight status, and elevated ARG abundance

To explore potential factors influencing ARG abundance, we conducted a detailed subgroup analysis (Fig. 5A). Participants over the median age of 36 exhibited a stronger association with ARG abundance (β = 0.27, 95% CI: 0.17–0.38, $p < 0.001$) compared to those aged 36 or below (β = 0.04, 95% CI: −0.10–0.19, $p = 0.57$), with a significant interaction effect (*p* for interaction = 0.01). Additionally,

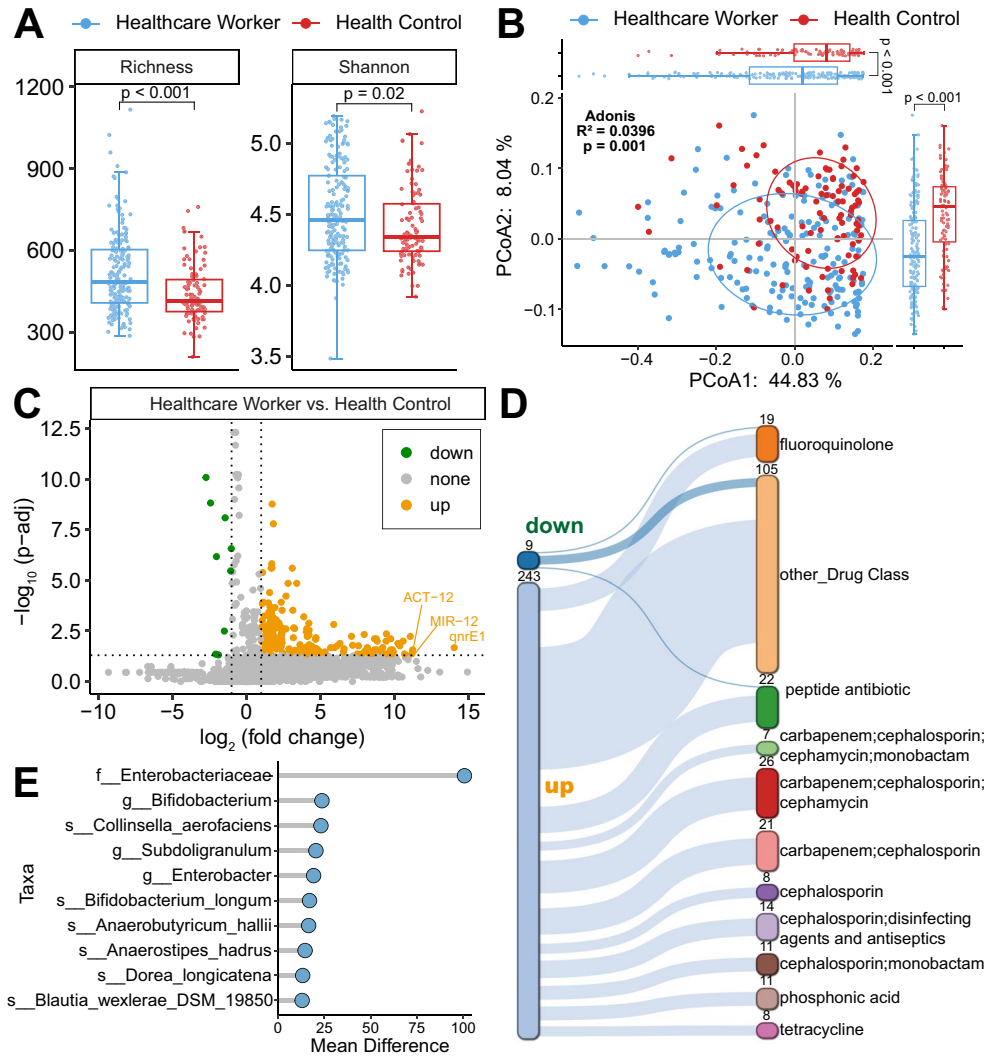

Fig. 3 The composition of ARGs between healthcare workers and health controls. **A** Alpha diversity of ARGs is assessed using Richness and Shannon indices, both indicating significantly higher diversity in healthcare workers compared to health controls (Richness: $p < 0.001$; Shannon: $p = 0.02$). The box plots show each group's median (horizontal line) and IQR (box edges), with whiskers representing 1.5 times the IQR. **B** Principal coordinate analysis (PCoA) of β-diversity based on Bray–Curtis dissimilarity demonstrates a clear separation of ARG composition between healthcare workers and health controls (Adonis $R^2 = 0.0396$, $p = 0.001$). Each point represents a sample, and the ellipses represent each group's 95% confidence interval. The box plots on the margins of the PCoA axes represent the distribution of ARG composition differences along each principal coordinate (PCoA1 and PCoA2) between healthcare workers and health controls. **C** Volcano plot showing the differential abundance of ARGs between healthcare workers and health controls. Yellow dots indicate ARGs significantly upregulated in healthcare workers (log2 fold change > 1, *p*-adj < 0.05), while green dots represent significantly downregulated ARGs. **D** The Sankey diagram represents the relationship between differentially abundant ARGs and their corresponding antibiotic classes. The majority of upregulated ARGs in healthcare workers are associated with fluoroquinolone and carbapenem resistance. **E** The upregulated ARGs in healthcare workers are primarily contributed by bacteria such as *f_Enterobacteriaceae*, *g_Bifidobacterium*, and *s_Collinsella aerofaciens*. The mean difference refers to the difference in the average abundance of each bacterial host between the two groups. The greater the mean difference is, provided the statistical *p*-value for this difference is less than 0.05, the more significant that bacterial species contributes to the increased ARG abundance observed in healthcare workers

the interaction effect for BMI was also significant (p for interaction = 0.02). Participants with a BMI greater than 24 showed a significant increase in ARG abundance (β = 0.33, 95% CI: 0.21–0.45, $p < 0.001$), whereas the associations for the underweight (< 18) and normal BMI (18–24) groups were not significant. However, in the BMI-normal subgroup, β-diversity analysis revealed a significant difference in the ARG composition between healthcare workers and health controls (Adonis $R^2 = 0.0261$, $p = 0.009$) (Figure S1). Gender presented a marginal interaction effect

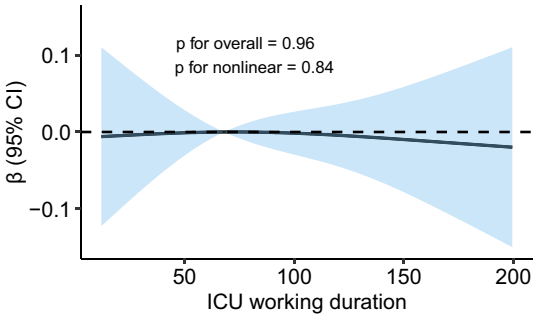

Fig. 4 Association between the duration of ICU work and ARG abundance in healthcare workers. The restricted cubic spline (RCS) plot demonstrates the relationship between ICU working time and log-transformed ARG abundance. The black curve represents the estimated β coefficient, and the shaded area indicates the 95% confidence interval. The analysis reveals no significant linear or nonlinear relationship between ICU working duration and ARG abundance (*p* for overall = 0.96, *p* for nonlinear = 0.84). BMI, age, and gender have been adjusted as covariates

(p for interaction = 0.07), with males showing a stronger association with ARG abundance (β = 0.29, 95% CI: 0.18–0.40, $p < 0.001$) compared to females (β = 0.13, 95% CI: 0.01–0.25, $p = 0.03$). No significant interaction effect was observed for underlying disease status (p for interaction = 0.69). But in the subgroup without underlying diseases, β-diversity analysis revealed a significant difference in the ARG composition (Adonis $R^2 = 0.0437$, $p = 0.001$) (Figure S2).

Sensitivity analysis confirmed that the total ARG abundance remained consistently higher in healthcare workers

To further evaluate the robustness of our findings, we conducted sensitivity analyses. Healthcare workers from Henan province exhibited higher total ARG abundance (fold change = 1.20, 95% CI: 1.16–1.46, $p < 0.001$) compared to those from Zhejiang province (fold change = 1.12, 95% CI: 1.07–1.30, $p = 0.001$) (Fig. 5B). Among different medical professions, nursing assistants showed the highest total ARG abundance (fold change = 1.33, 95% CI: 1.29–1.76, $p < 0.001$), followed by physicians (fold change = 1.18, 95% CI: 1.09–1.47, $p = 0.002$) and nurses (fold change = 1.08, 95% CI: 1.01–1.23, $p = 0.03$) (Fig. 5C). The β-diversity analysis consistently demonstrated that the ARG composition of healthcare workers was more complex than that of health controls across all comparisons ($p < 0.05$), including different provinces and medical professions (Figure S3-S4).

Significant differences in gut bacterial composition between groups

Leveraging metagenomic technology, we are also able to report additional differences in the gut microbiome between the two groups. Alpha diversity showed significantly higher bacterial richness and Shannon index in healthcare workers compared to health controls ($p = 0.04$ and $p = 0.03$, respectively) (Figure S5 A). Beta diversity indicated a clear difference in bacterial composition between the two groups (Adonis $R^2 = 0.0419$, $p = 0.001$) (Figure S5B). A total of 95 bacterial species were significantly upregulated in healthcare workers (Figure S5 C). The most upregulated species included *Klebsiella grimontii* (log2 fold change = 5.93, *p*-adj = 0.004), *Lactococcus raffinolactis* (log2 fold change = 5.78, *p*-adj = 0.009), and *Klebsiella michiganensis* (log2 fold change = 3.03, *p*-adj < 0.001). However, there were no significant differences in viral composition between or within healthcare workers and health controls in alpha diversity (Richness: $p = 0.25$, Shannon: $p = 0.27$) (Figure S6 A) or beta diversity (Adonis $R^2 = 0.0057$, $p = 0.09$) (Figure S6B). The volcano plot also showed no significant differential abundance of viral species (Figure S6 C).

Discussion

We conducted a multicenter study across two provinces in China, involving eight medical centers, and found that gut ARG abundance in ICU healthcare workers was significantly higher than in healthy controls. This suggests that occupational exposure in healthcare settings may contributes to elevated ARG levels in the gut microbiome of healthcare workers. Besides, we did not find a statistically significant link between ICU work duration and ARG abundance, suggesting that ARG exposure may begin early in healthcare careers.

Previous studies have shown that ICU air has higher ARG concentrations than external environments, and surfaces in healthcare facilities are colonized by MDROs, making occupational exposure nearly unavoidable [4, 16]. Elevated ARGs in healthcare workers primarily target antibiotics like fluoroquinolones and carbapenems, commonly used in ICUs and linked to multidrug resistance [17]. ICU healthcare workers may acquire antimicrobial-resistant pathogens through airborne transmission, contact with contaminated surfaces, or direct patient contact, as ICU patients often harbor MDROs. The high antibiotic usage and pathogen load in ICU settings likely exert selective pressure on bacteria, altering the gut microbiome of healthcare workers [18–20].

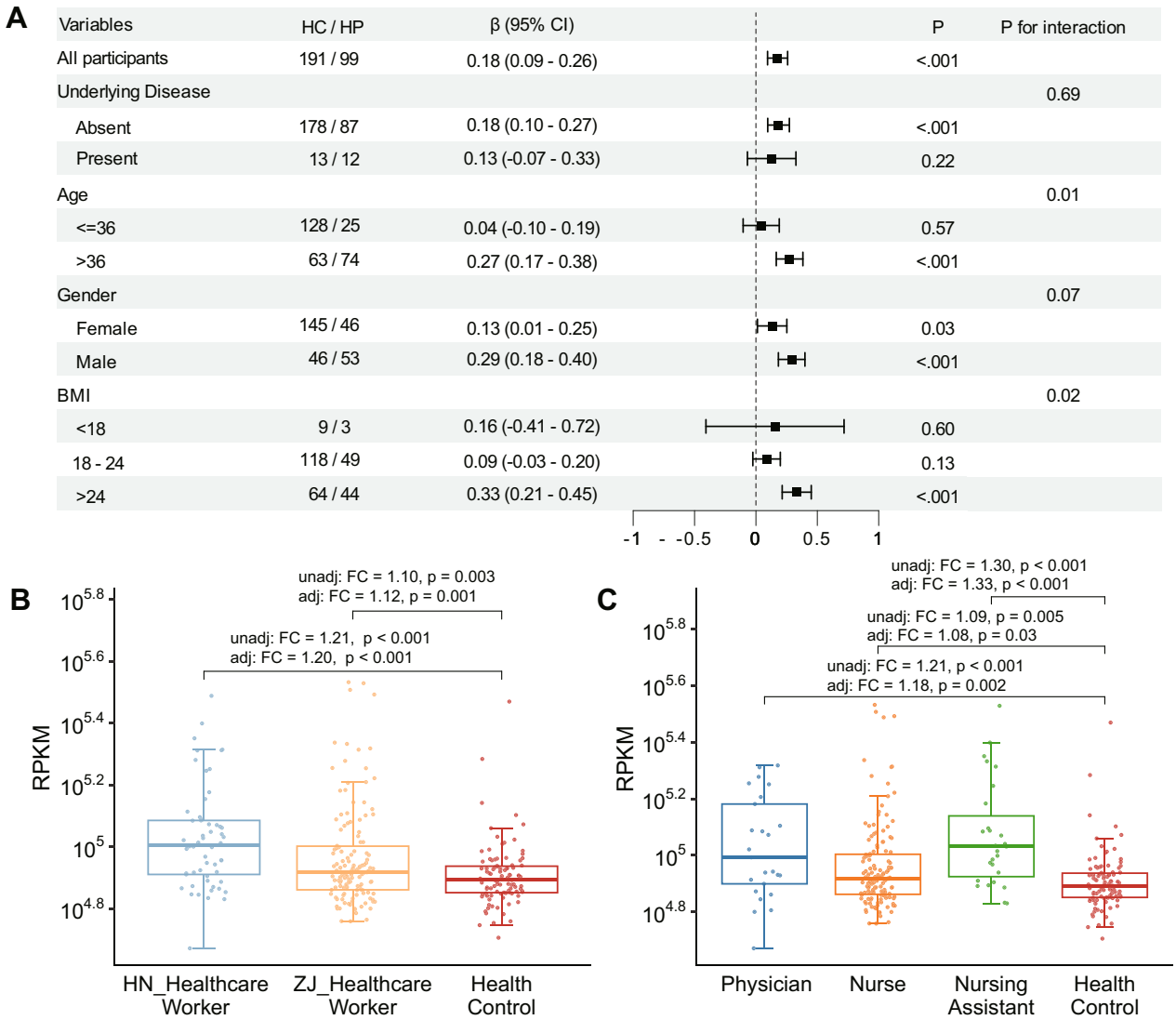

Fig. 5 Subgroup and sensitivity analyses of ARG abundance in healthcare workers and health controls. **A** Subgroup analysis of ARG abundance compared healthcare workers (HW) and healthy population (HP) across different variables. Regression coefficients (β), 95% confidence intervals (CI), and *p*-values are displayed. Significant interaction effects are observed for age (*p* for interaction = 0.01) and BMI (*p* for interaction = 0.02), with higher age and BMI > 24 being associated with increased ARG abundance among healthcare workers compared to health controls. **B** Sensitivity analysis compared the abundance of ARG between healthcare workers from the Henan (HN) and Zhejiang (ZJ) provinces and health controls. Both Henan and Zhejiang healthcare workers exhibit significantly higher ARG abundance compared to health controls (fold change = 1.20, 95% CI: 1.16–1.46, $p < 0.001$ and fold change = 1.12, 95% CI: 1.07–1.30, $p = 0.001$, respectively, adjusted by age, gender, and BMI). **C** Sensitivity analysis compared ARG abundance between different medical professions and health controls. Nursing assistants have the highest ARG abundance (fold change = 1.33, 95% CI: 1.29–1.76, $p < 0.001$), followed by physicians (fold change = 1.18, 95% CI: 1.09–1.47, $p = 0.002$) and nurses (fold change = 1.08, 95% CI: 1.01–1.23, $p = 0.03$). BMI, age, and gender have been adjusted as covariates

While this study focuses on identifying differences in ARG abundance between ICU healthcare workers and healthy individuals, our future research will incorporate a well-designed longitudinal cohort to investigate the key ecological exposures contributing to ARG enrichment using our self-developed exposure group sampling devices [21–23]. Besides, we will report whether there are transmission of ARGs from healthcare workers to patients when we finish our prospective cohort [24].

ESKAPE pathogens, including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp., are widely recognized for their antibiotic resistance and pose significant threats to

public health [25, 26]. Previous studies have shown that residents in nursing homes have significantly elevated ARGs on their skin, originating from ESKAPE pathogens [26]. In our study, we found that the increase in ARGs in the gut of ICU healthcare workers primarily originated from Enterococcus faecium, Klebsiella pneumoniae, and Enterobacter spp., while the abundance of ARGs from other ESKAPE pathogens was similar to that found in healthy populations. This suggests that the elevation of ARGs in the gut of healthcare workers may be strain-specific, potentially related to the varying horizontal gene transfer (HGT) efficiency between different species.

Our study also revealed differences in gut microbiota composition between healthcare workers and healthy controls. The ICU environment is one of the most conducive settings for pathogen transmission, where healthcare workers are frequently exposed to bacterial, viral, and fungal infections [27]. Notably, Klebsiella oxytoca complex abundance increased among healthcare workers, with Klebsiella grimontii and Klebsiella michiganensis showing the most significant increase. These bacteria are known for ARG transmission via horizontal gene transfer (HGT), and their increased abundance may reflect either acquired multidrug-resistant traits or adaptive responses to ARG exposure [28–30].

Our study has several limitations. First, it exclusively included a Chinese population, which may limit the generalizability of our findings. Second, we did not perform stool cultures to confirm MDRO colonization. Third, as an observational study, we cannot establish a definitive causal relationship between occupational exposure and the increase in ARG abundance. While we accounted for key confounders, unmeasured factors such as prior travel, previous hospitalization, and alcohol-based hand rub use may still present limitations. In addition, although the healthy controls were recruited from individuals undergoing routine health check-ups and were screened based on strict exclusion criteria, we acknowledge that residual unmeasured confounding might still exist due to differences in background exposure and lifestyle. However, such bias is largely inevitable in real-world studies and is unlikely to fully account for the widespread elevation of ARG abundance observed in ICU healthcare workers. Lastly, the clinical relevance and Infection Prevention and Control implications remain uncertain, as many non-pathogenic strains carry ARG, with unclear infection risks, transmission dynamics, and carriage duration. Therefore, the conclusions of this study should be interpreted with caution.

Despite these limitations, our study has several notable strengths, including its prospective, multicenter design and the use of metagenomic analysis instead of traditional culture-based methods. We also conducted multiple sensitivity analyses, which consistently demonstrated that healthcare workers had higher gut ARG abundance compared to healthy controls. Additionally, the use of metagenomic techniques enabled us to identify further differences in the gut microbiome between healthcare workers and healthy controls. Whether these microbiome differences result from occupational exposure and how they may affect the health of healthcare workers remain key questions for our future research. Overall, our study provides new insights into the fields of occupational exposure, healthcare-associated infections, and antibiotic resistance and highlights the necessity of using metagenomic methods for research in related fields.

Conclusion

In this prospective, multicenter study, we found that ICU healthcare workers exhibit significantly higher gut ARGs abundance compared to healthy controls. Meanwhile, healthcare workers, including physicians, nurses, and nursing assistants, have a different composition of gut ARGs compared to healthy individuals.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13054-025-05408-5.

Supplementary material 1.

Supplementary material 2.

Supplementary material 3.

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Author contributions

CJ, LTH, HLC, GSZ designed the study. CJ, KCL, CP performed statistical analysis of the data. LTH, KCL, CP, XHH, CHG made the analysis. LTH, KCL, CP, SLG, XHH drafted the manuscript, prepared the figures and critically reviewed the final manuscript. HLC, CHG, XDR, MHC, YHX, YPJ, HYW, MQW, PS, QQW, XWH, LZ, SFW, NZ participated in enrollment. All authors contributed to the article and approved the submitted version.

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Data availability

All sequencing data from this study, after quality control and host removal, have been uploaded to the European Nucleotide Archive (PRJEB79317).

Declarations

Ethics approval and consent to participate
The study has been approved by the ethics committees of Zhejiang University School of Medicine First Affiliated Hospital in accordance with the Declaration of Helsinki (IIT20230455B).

Consent for publication
Informed consents were obtained from all participants and all participants agreed to publish this study.

Competing interests
The authors declare no competing interests.

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