Gut Pathogens

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Contrasting the gut microbiome in Colombian patients with diarrhea: a comparative metagenomic study in hospitalization and emergency room services

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Abstract

Background Diarrhea remains a major cause of morbidity worldwide, particularly in low- and middle-income countries. Hospital environments impose strong selective pressures on the gut microbiome through antimicrobial exposure, invasive procedures, and pathogen transmission, yet differences between hospital-onset and community-onset diarrhea remain poorly characterized at the microbiome level. This study aimed to compare the taxonomic and functional profiles of the gut microbiome in hospitalized (Hosp) and emergency room (ER) patients with diarrhea using shotgun metagenomics.

Results Fecal samples from 41 patients (Hosp = 24; ER = 17) attending the Hospital Universitario Mayor–Méderi (Bogotá, Colombia) were analyzed. The gut microbiomes were dominated by Enterobacteriaceae, particularly *Klebsiella pneumoniae* and *Escherichia coli*, together with abundant bacteriophages from the families Myoviridae, Siphoviridae, Podoviridae, and crAss-like phages. Phages predicted to infect *Escherichia* and *Klebsiella* were significantly depleted in Hosp patients ($p < 0.05$). Read-based functional profiling revealed the presence of virulence factors associated with *K. pneumoniae* capsule biosynthesis, secretion systems, and toxins from *Clostridioides difficile* and *Clostridium perfringens*. In parallel, Hosp patients showed a higher diversity of antimicrobial resistance markers, with a marked increase in glycopeptide resistance determinants. A total of 492 high-quality metagenome-assembled genomes were reconstructed, including multiple diarrhea-associated taxa. Hosp patients exclusively harbored genomes of *K. pneumoniae*, *Enterococcus faecium*, and most reconstructed *Clostridium* species (*C. symbiosum*, *C. saccharolyticum*_A, *C. innocuum*, *C. scindens*, *C. leptum*, and *Clostridium* sp000435835). In contrast, ER patients harbored genomes classified as *Escherichia coli*, *Escherichia flexneri*, and *Enterococcus faecalis*. Genomes associated with hospitalization carried higher loads of antimicrobial resistance markers (e.g., *oqxA* and *aac(6')-Ii*) and virulence factors (e.g., *iutA* and *traT*), whereas ER genomes, particularly *E. coli* and *E. flexneri*, encoded diverse aminoglycoside resistance and adhesion traits.

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Conclusions Hospital-onset diarrhea was associated with distinct microbiome features, including differences in phage–bacteria dynamics involving key diarrhea-associated taxa, as well as a higher abundance of virulence factors and antimicrobial resistance markers. These findings underscore the potential value of shotgun metagenomics as a complementary approach for infection surveillance and the development of precision diagnostic strategies in hospital settings.

Keywords Gut microbiome, Hospital-associated infections, Shotgun metagenomics, Virulence factors (VFs), Antibiotic resistance markers (ARMs)

Background

In hospital settings, patients are exposed to multiple risk factors that can disrupt baseline health status and significantly alter the composition of their gut microbiome. Prominent among these risks are exposure to pathogens, transmission of microorganisms from the environment or medical personnel, invasive procedures, use of medical equipment, and the administration of medications, particularly antibiotics [1–3]. Such exposures constitute a major public health concern, as they contribute to the development of healthcare-associated infections, especially in critically ill patients [4, 5]. These infections complicate clinical outcomes and increase the burden on healthcare systems. Numerous studies have shown that the gut microbiome of hospitalized patients, whether in general wards or intensive care units (ICUs), undergoes marked shifts in community composition, frequently characterized by an increased abundance of opportunistic and potentially pathogenic microorganisms [6–8]. Importantly, acute diarrhea acquired in the community remains a major cause of morbidity globally, frequently driven by enteric bacterial pathogens with high levels of antimicrobial resistance. These community-onset infections represent an important public health issue and an epidemiological reservoir that can introduce clinically relevant pathogens and resistance determinants into hospital environments, especially through patient admissions and healthcare interactions [9]. Together, these observations highlight the importance of understanding microbiome dynamics across the community–hospital interface to mitigate health risks and improve patient outcomes.

Shotgun metagenomics provides a comprehensive, culture-independent approach for microbiome characterization, enabling the simultaneous generation of high-resolution taxonomic and functional profiles across diverse biological samples. This methodology has transformed microbiome research by allowing genome reconstruction through metagenome-assembled genomes (MAGs), which provide insights into microbial diversity and genomic functionality [9, 10]. Metagenomic approaches have been widely applied to identify circulating pathogens in hospital environments and patient samples, often in association with healthcare-associated infections and adverse clinical outcomes [11–13]. The high resolution achieved by shotgun metagenomics enables strain-level profiling and genotyping of clinically relevant organisms, such as *Klebsiella pneumoniae*, thereby supporting real-time pathogen surveillance and molecular epidemiology in hospital settings [14, 15]. Moreover, this approach facilitates the exploration of the gut virome, a component of the microbiome that remains comparatively underexplored. Integrating microbial communities provides a more complete view of the ecological interactions and functional potential of the gut microbiome and its influence on host health [16–19].

The gut virome comprises a diverse array of single-stranded and double-stranded DNA and RNA viruses, although most current knowledge is centered on DNA bacteriophages [17, 20]. These viral members play a crucial role in modulating the microbiome through trans-domain interactions, impacting host metabolism and immune responses. Alterations in virome composition have been reported in the progression of several diseases [17, 21–25]. For instance, studies in colorectal cancer, ulcerative colitis, and *Clostridioides difficile* infection (CDI) have described increased phage abundance in the gut microbiome, frequently associated with complex phage–bacteria dynamics such as prophage induction and lytic cycling [21, 22, 26]. Despite their relatively low abundance compared with bacteria, viral members can exert substantial effects on microbial community structure and patients' health, underscoring the relevance of investigating these communities.

Beyond taxonomic profiling, shotgun metagenomics enables in-depth functional characterization of the gut microbiome, including the identification of virulence factors (VFs) and antibiotic resistance markers (ARMs) [27, 28]. These molecular markers are central to microbial adaptation in hospital environments, where strong selective pressures imposed by disinfectants, medications, and antibiotics drive the evolution of multiple survival strategies [5]. Several studies have identified bacterial VFs and ARMs associated with diseases such as inflammatory bowel disease, cirrhosis, and obesity [29–31]. Globally, the rise of antibiotic resistance is a growing concern, driven by factors such as human–environment microbial transmission and the indiscriminate use of antibiotics [32]. For instance, VFs such as the *tcdA* and *tcdB* toxins of *Clostridioides difficile*, the capsule-associated genes

of *Klebsiella pneumoniae*, as well as factors related to biofilm formation and bacterial attachment, including adhesins that bind to sialic acid receptors such as F1C fimbriae, S fimbriae, and the porcine attaching and effacing–associated adhesin (Paa) [29], have been directly linked to host colonization, immune evasion, and disease severity. Likewise, antimicrobial resistance markers such as *aac*(6')-*Ii*, *oqxA*, and extended-spectrum β-lactamase–associated genes are frequently reported in hospital-associated pathogens and compromise the efficacy of first-line therapies [32].

Shotgun metagenomics also represents a powerful approach for detecting ARMs in hospital settings, where antibiotic-resistant infections are highly prevalent [33]. For instance, Herrera et al. (2022) applied this methodology to characterize the gut microbiome of Colombian patients with *Clostridioides difficile*-associated diarrhea and identified numerous ARMs conferring resistance to fluoroquinolones and aminoglycosides [34]. Similarly, recent studies have revealed associations between microbial community composition and resistance profiles in hospitalized patients. Bai et al. (2025) reported a positive correlation between the abundance of *Klebsiella pneumoniae* and the presence of fosfomycin or multidrug resistance genes in patients with acute gastrointestinal injury who did not survive their ICU stay [35]. Similarly, Wang et al. (2025) characterized the microbiome of a cohort of patients with *Clostridioides difficile* and reported a high abundance of *Klebsiella*. Their results suggested that the dominance of *Klebsiella* contributed to resistance against acriflavine and glycylcycline [36]. Advancing such analyses is critical to better understanding the dynamics of microbial adaptation and mitigating associated health threats.

Despite the global expansion of microbiome research, substantial knowledge gaps persist in Latin American countries such as Colombia, particularly regarding the gut microbiome in hospital settings. Microbial diversity, especially within the viral community, as well as the distribution of antimicrobial resistance markers (ARMs) and virulence factors (VFs), remains poorly characterized. In this study, shotgun metagenomics was applied to characterize the gut microbiome of patients with diarrhea attending Hosp and ER services at Hospital Universitario Mayor–Méderi (Bogotá, Colombia). The composition of prokaryotic microbial communities (bacteria and archaea) and the DNA gut virome was characterized, while the presence of virulence factors and antimicrobial resistance markers was assessed in parallel. By providing an integrated taxonomic and functional characterization of hospital-onset and community-onset diarrhea, this work contributes to a better understanding of microbiome alterations in hospital environments. Furthermore, our findings may inform the future development of rapid molecular diagnostic tools and support improvements in patient care and infection control strategies.

Methods

Ethical considerations

The study was conducted with approval from the Research Ethics Committee of Universidad del Rosario (Approval code: DVO005 1856-CV1493). Classified as low risk under Resolution 8430 of 1993 by Colombia's Ministry of Health, it posed no direct health hazard to participants. Samples were coded to ensure patient anonymity and informed consent was obtained in line with ethical guidelines. Clinical information databases were anonymized, with the Centro de Investigaciones de Méderi (CIMED) responsible for their curation and preservation, adhering to ethical research practices.

Patient recruitment and sample collection

Patient recruitment took place between August 2022 and May 2023. Enrolled subjects comprised adult patients (≥ 18 years) attending the Universitario Mayor Méderi in Bogotá, Colombia. The main inclusion criterion was the presence of diarrhea, defined as more than three unformed stools in a 24-h period. All participants voluntarily agreed to participate and signed the informed consent prior to enrollment. Patients with HIV/AIDS, cancer, SARS-CoV-2 infection, colitis, ulcerative colitis, Crohn's disease, colorectal cancer, intestinal or digestive tract obstructions, or protein-calorie malnutrition were excluded to minimize potential confounding effects. Table S1 (Additional file 1) summarizes the clinical information collected. Patients were classified into two categories based on the timing of diarrhea onset relative to hospital admission, in accordance with international clinical practice guidelines for adult CDI (Cohen, et al., 2010): Emergency room (ER) patients ($n = 17$) were defined as individuals who presented with diarrhea at admission or within the first 72 h after arrival at the ER triage area, consistent with community-onset diarrhea. Hospitalized (Hosp) patients ($n = 24$) were defined as individuals who developed diarrhea ≥ 72 h after admission to an inpatient hospital ward, consistent with healthcare-associated (nosocomial) diarrhea. This group included patients admitted to the Intensive Care Unit (ICU), a population at increased risk due to higher susceptibility and complication rates associated with CDI. Their inclusion provides insight into the clinical complexity and pathophysiological mechanisms of diarrhea in critically ill patients (Karanika, et al., 2016).

Fecal sample processing, DNA extraction and sequencing

Diarrheic fecal samples were collected in sterile containers without transport media, transferred to sealed plastic bags under biosafety conditions, and stored under

refrigeration (2–8 °C) until processing within 72 h of collection. Prior to DNA extraction, samples were homogenized by mechanical disruption using sterile disposable spatulas. Approximately 200 mg of stool material was used as input for each DNA extraction, which was performed using the Stool DNA Isolation Kit (Norgen Biotek Corp., Thorold, Canada), following the manufacturer's protocol. DNA was eluted in 40 µL of the provided elution buffer. Subsequently, an initial quality control step was performed by evaluating DNA quantity and purity using a NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). DNA integrity was assessed by 1.5% agarose gel electrophoresis; only samples showing a single, non-degraded band were considered for sequencing. DNA was subsequently stored at − 20 °C and shipped to Novogene (Sacramento, California, USA) on dry ice via an internationally certified carrier. At the sequencing facility, DNA integrity was reconfirmed using a Fragment Analyzer (Agilent Technologies) to ensure single-peak profiles, and fluorometric quantification was performed to verify nucleic acid concentration. Sequencing was performed at Novogene (Sacramento, CA, USA), where Shotgun Metagenomics Libraries were prepared following Illumina-recommended protocols. Libraries were normalized and pooled according to the required sequencing depth, and sequencing was conducted on the Illumina NovaSeq 6000 platform using paired-end 150 bp reads. A minimum output of 6 Gb of raw data per sample was ensured under Novogene's standard WOBI workflow for shotgun metagenomics.

Read-based analysis of metagenomic data

Initially, the sequence quality of each sample was assessed with FastQC v.0.11.9 [37] and MultiQC v.1.6 [38]. Afterward, Trimmomatic v.0.38 was employed to filter the reads based on the following criteria: MINLEN:150, AVGQUAL:20, and activating the "ILLUMINACLIP" parameter to remove the adapters from the reads using TrueSeq3-PE sequences [39]. On average, only 2.16% of reads were dropped in this filtering process. The filtered reads were aligned against the NCBI-reported human genome (Genome Reference Consortium Human Build 38 [GRCh38], accession number PRJNA31257) using Bowtie2 (v2.4.4) [40] to remove all possible host reads. Taxonomic assignment of clean reads was performed with Kraken2 v2.0.7-beta employing the PlusPF database [41]. The obtained reports were visualized and manipulated using the Pavian web interface [42]. Additionally, the GraPhlAn tool [43] was used to generate a cladogram of the predominant taxa for each group (Fig. 1) based on reports generated by Kraken2.

The R package *phylose* was used to import, analyze, and generate graphical representations of the sample's microbiome composition. Taxonomic read counts were converted to relative abundances by dividing each taxon's count by the total number of reads in the respective sample, yielding compositional profiles suitable for comparative microbiome analyses (Xia et al., 2023). Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) [44] was performed to identify differentially abundant bacterial and viral species between the two study groups (Hosp vs. ER). ANCOM-BC was computed by using read counts of the 1,000 most abundant species to reduce sparsity and multiple testing burden. Additionally, we applied a prevalence cutoff of 10% (pvr_cut = 0.10) and the Holm-Bonferroni method to adjust p-values for multiple testing with a significance threshold of 0.05 (alpha = 0.05). Overall, taxonomic read counts were converted to relative abundances for descriptive analyses and visualization of community composition. Differential abundance testing was performed exclusively on raw read counts using ANCOM-BC, which explicitly accounts for compositionality and sequencing depth heterogeneity. Figure construction and statistical analyses were performed using the R v.4.4.1 software, along with *vegan*, *phyloseq*, *reshape2*, *rstatix*, *tidyverse*, and *ggplot2* packages (R Core Team, Vienna, Austria). The raw data underlying the taxonomic profiling are provided in Table S2 (Additional file 2).

Identification of virulence factors and antibiotic resistance markers

The Resistance Gene Identifier (RGI) was used to identify ARMs. Quality-controlled reads were used as input for RGI, with the Comprehensive Antibiotic Resistance Database (CARD, version 3.1.3) [45] as the reference database. The *rgi bwt* option was used with the following parameters: –aligner bowtie2 –include_wildcard –local. Identified ARMs were categorized as chromosome-associated or plasmid-associated according to the curated metadata provided by CARD, which reflects the typical genomic context reported for each resistance gene in reference genomes. This classification does not represent direct inference of the physical genomic location of ARMs. For downstream comparative analyses between Hosp and ER patient groups, ARMs were grouped by antibiotic class. For each sample, read counts mapping completely to ARMs within each antibiotic class were summed and normalized by the total number of mapped reads in the corresponding sample, yielding relative abundances that account for differences in sequencing depth across samples before statistical analysis described in the read-based analysis of metagenomic data section.

Identification of VFs was conducted using the Basic Local Alignment Tool (BLASTn) [46]. Decontaminated reads were aligned against the Virulence Factors Database (VFDB) [47], considering a minimum percentage of

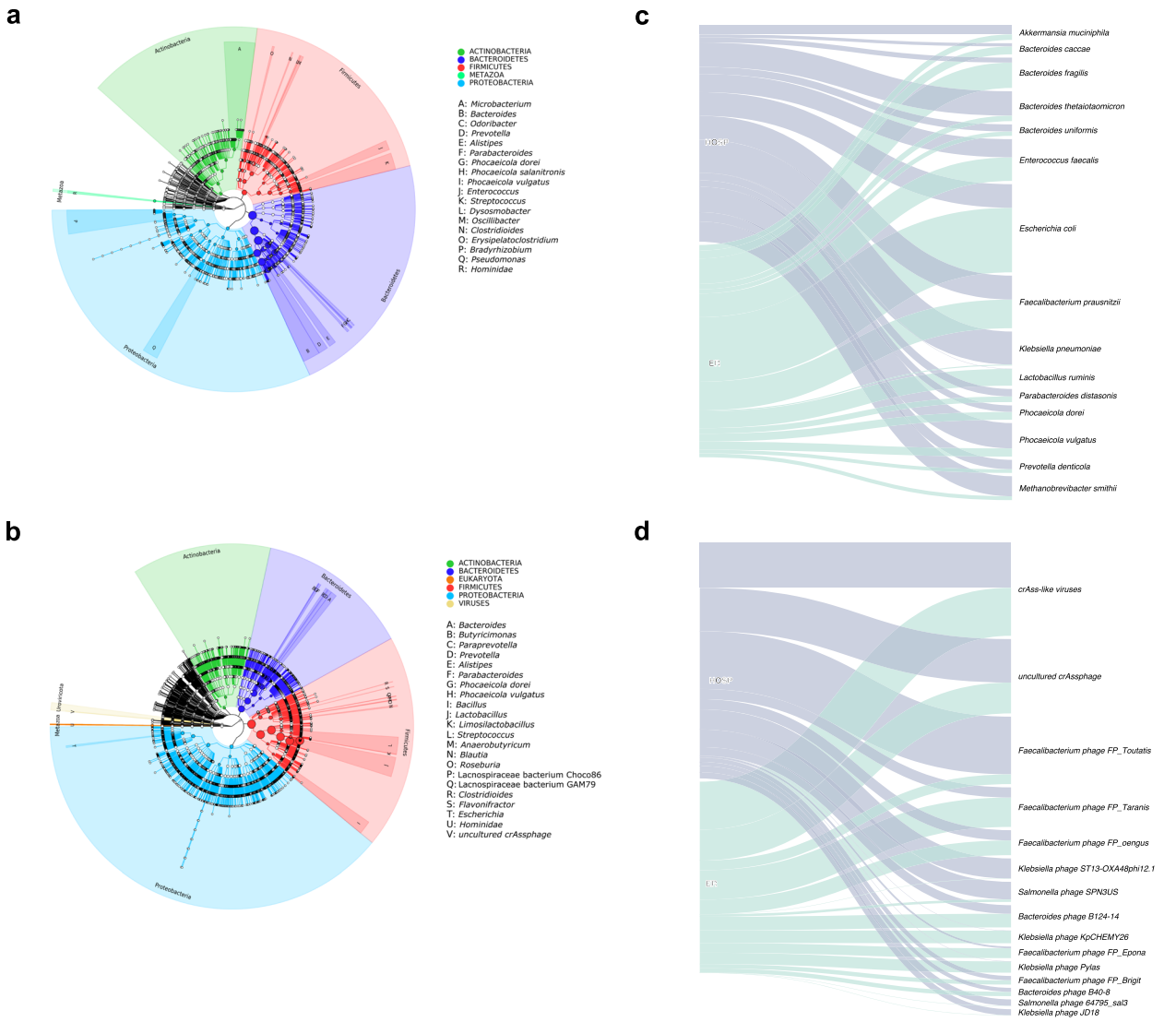

Fig. 1 Gut microbiome profiling of the patients belonging to hospitalized (Hosp) and emergency room (ER) groups. **a.** Cladogram of the predominant taxa identified in Hosp patients. **b.** Cladogram of the predominant taxa identified in ER individuals. **c.** Sankey plot displaying the mean relative abundance of the top 10 microbial species in the study groups. **d.** Sankey plot displaying the mean relative abundance of the top 10 viral species in the study groups

identity of 95%. Identified VFs were then categorized into biofilm formation, effector delivery system, immune modulation, exotoxin, and exoenzyme categories. Only VFs associated with bacterial species of clinical relevance were retained for downstream analyses [48–50]. Read counts were normalized to relative abundances by dividing the number of mapped reads per VF category by the total number of mapped reads per sample. These normalized values were used for descriptive comparisons between study groups, while inferential analyses were performed using compositionality-aware methods as described in the read-based analysis of metagenomic data section. Graphical representations were generated using the R package *ggplot2*.

Assembly-based bioinformatic analysis of patients' metagenomes

De novo assembly of the clean reads was performed with metaSPAdes (v3.15.4) [51]. The clean reads were mapped to contigs using Bowtie2 (v2.4.4) and SAMtools (v.1.17) [52] to obtain the input files for the binning process. Maxbin (v.2.2.7) [53], Metabat2 (v.2.15) [54], and CONCOCT (v.1.1.0) [55] were employed for contig binning, and DASTool (v.1.7.0) [56] was used for refining the binning for each sample. The quality of these MAGs was assessed using CheckM (v.1.1.3) [57]. MAGs were classified as high- (HQ), medium- (MQ), or low- quality (LQ) according to the MIMAG criteria proposed by Bowers et al. (2017) [58]. Specifically, HQ MAGs were defined as having ≥ 90% completeness and ≤ 5% contamination, MQ MAGs as having ≥ 50% completeness

and ≤ 10% contamination, while MAGs not meeting these thresholds were classified as LQ. Only HQ MAGs were selected for downstream analyses (n = 492). Taxonomic assignment of HQ MAGs was performed with GTDB-Tk (v.1.7.0) [59], using default parameters.

The PhyloPhlAn pipeline (v.3.0.67) [60] was used for phylogenetic reconstruction of the HQ MAGs by employing 400 universal markers from the PhyloPhlan database and the following parameters: –diversity medium and –min_num_markers 100. The reconstructed tree was visualized and edited on Interactive Tree of Life (iTOL) [61]. Once the taxonomic assignment of the MAGs was established, we selected those corresponding to clinically relevant diarrhea-associated bacterial species for downstream analysis. Specifically, MAGs belonging to *Clostridium* spp., *Campylobacter jejuni*, *Escherichia flexneri, Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Klebsiella pneumoniae*, were included, given their established roles in gastrointestinal infections and their relevance to the Hosp and ER settings studied.

Functional annotation of metagenome-assembled genomes

MAGs belonging to the selected taxa were dereplicated using the *dereplicate* function in dRep [62] under default parameters to obtain a single representative genome per species**.** These representative MAGs were annotated with Prokka (v.1.14.5) to generate general genomic features. Functional annotation of protein-coding sequences was performed using eggNOG-mapper (v.2.1.9) [63], employing default settings. Genes were analyzed according to the Cluster of Orthologous Genes (COGs) category to which they belonged.

To detect virulence factors (VFs) and antibiotic resistance markers (ARMs), the ABRicate pipeline [64] was employed, using the VFDB and CARD databases, respectively [45]. Identified virulence factors were categorized into the following functional classes: adherence, motility, invasion, exotoxins, immune modulation, and nutritional/metabolic factors. For both VFs and ARMs, only those VFs and ARMs with a percentage of identity higher than 95% were considered.

Results

A total of 41 were stratified according to diarrhea onset relative to hospital admission into emergency room (≤ 72 h, community-onset, ER = 17) and hospitalized (≥ 72 h, healthcare-associated, Hosp = 24) groups. DNA was extracted from fecal samples and subjected to shotgun metagenomic sequencing to characterize the composition of prokaryotic communities (bacteria and archaea) and the DNA gut virome, together with the intestinal repertoire of circulating VFs and ARMs.

Read-based taxonomic profiling of bacterial, archaeal, and viral communities

On average, each sample yielded 23,578,014 raw reads, of which 19,132,256 remained after Trimmomatic filtering and host sequence removal. The taxonomic classification (Additional file 2: Table S3) assigned 53.2% to 58.6% of input reads to specific taxa, primarily prokaryotic species, including both bacteria and archaea (97%–98.1%). The remaining percentage comprised viral reads (0.82% to 1.07%), while a minor fraction was attributed to fungi and protozoa (0.039%–0.051%). Given their minimal contribution, eukaryotic reads were excluded from the detailed analysis.

The mean number of reads was 20,973,006 (SD: 6,493,994) in the Hosp group and 16,533,551 (SD: 11,173,188) in the ER group. There was no statistically significant difference in read counts between the groups ($p > 0.05$) (Additional file 3, Fig. S1). Both groups exhibited dominance of Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Fig. 1a, Fig. 1b). The Hosp group was characterized by high relative abundances of Bacteroidetes (35.78%, SD: 29.18%) and Firmicutes (27.43%, SD: 21.19%), whereas ER patients had increased levels of Firmicutes (38.33%, SD: 28.98%) and Proteobacteria (29.45%, SD: 32.70%) (Additional file 3, Fig. S2a).. Viral community description revealed a dominance of Uroviricota, particularly crAss-like phages, which exceeded 90% abundance in both groups (Additional file 3, Fig. S2b).

Taxonomic analysis at the family level identified Enterobacteriaceae as predominant in both groups (mean: 24.03%, SD: 32.56%), followed by Bacteroidaceae (17.04%, SD: 19.84%) and Ruminococcaceae (8.61%, SD: 9.59%) (Additional file 3, Fig. S2c). Among viral families, Myoviridae (42.83%, SD: 31.48%), Podoviridae (24.90%, SD: 32.45%), and Siphoviridae (20.98%, SD: 27.45%) dominated across samples (Additional file 3, Fig. S2d)..

Figure 1c shows the relative mean abundance of the ten most abundant prokaryotic species across the study groups. *Methanobrevibacter smithii,* an archaeal species, was the only archaeon included in the top 10 with a relatively high abundance mean: 5.28%, SD: 13.94%. Although it was detected in both groups, its abundance was higher in hospitalized patients. Among bacterial species, *Escherichia coli* (mean: 16.99%, SD: 22.82%) was the most abundant in the ER group, while *Klebsiella pneumoniae* (mean: 8.69%, SD: 13.08%) was notable in the Hosp group. Figure 1d, by contrast, presents viral species, where crAss-like viruses were detected in both groups without significant differences in relative abundance.

Differential abundance analysis of read-based taxonomic profiles was performed using Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) to identify taxa significantly enriched or

depleted between the study groups. The analysis revealed a significant depletion of 11 viral species in the Hosp group relative to the ER group, as indicated by negative log-fold change (LFC) values. Notably, several phages predicted to infect *Klebsiella* and *Escherichia* were among the depleted taxa in Hosp patients (Fig. 2). In contrast, no bacterial or archaeal species met the criteria for statistical significance in the ANCOM-BC analysis. This observation is consistent with the results presented in Fig. 1c, where apparent differences in the relative abundance of specific prokaryotic taxa (e.g., *M. smithii*, *E. coli*, *K. pneumoniae*) did not reach statistical significance ($p > 0.05$) and were not identified as differentially abundant by ANCOM.

Read-based functional profiling of virulence factors (VFs)

Across all samples, 14,027 VFs were identified from read-based analyses by aligning quality-filtered reads against the VFDB. These included 1,636 VFs associated with biofilm formation, 8,662 with effector delivery systems, 2,316 with immune modulation, 879 with exotoxins, and 534 with exoenzymes. Taxonomic assignment of VFs was inferred based on the best-hit taxonomic annotation of the corresponding reads, identifying 25 bacterial taxa (Additional file 2: Table S4). Among these, 61 VFs from nine bacterial species of clinical relevance exhibited significant differences in relative abundance between groups ($p < 0.05$), as shown in Fig. 3. After collapsing duplicated annotations, these hits corresponded to 1,510 unique VF genes, many of which are shared across multiple bacterial species and strains. These unique VFs were grouped into five functional categories, including biofilm formation (1,636 hits), effector delivery systems (8,662 hits), immune modulation (2,316 hits), exotoxins (879 hits), and exoenzymes (534 hits). Most of these VFs associated with immune modulation ($n = 19$) and effector delivery

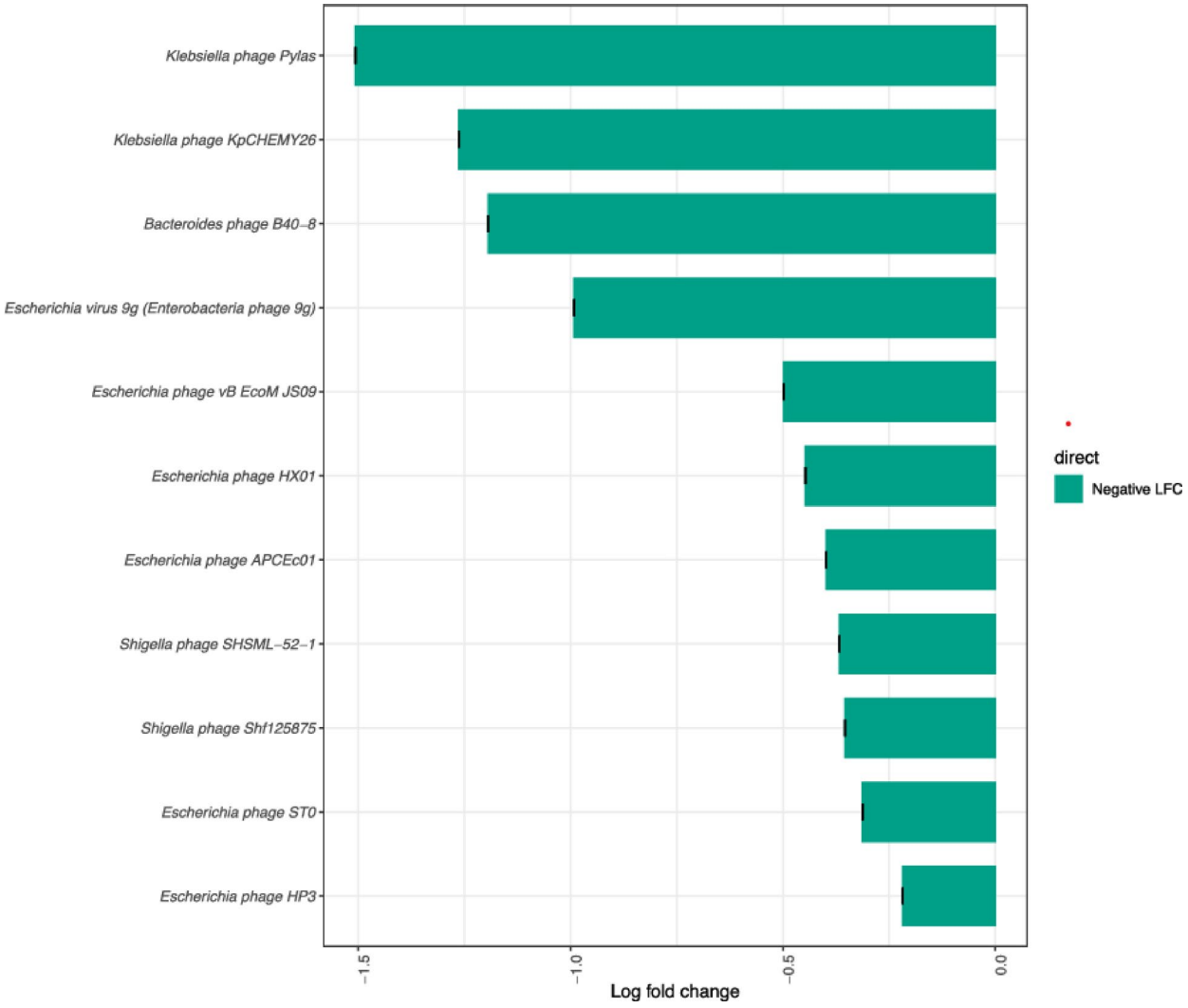

Fig. 2 Differential abundance of bacteriophages between hospitalized (Hosp) and emergency room (ER) groups. The Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) revealed 11 viral taxa depleted in Hosp patients in contrast with ER patients ($p < 0.05$)

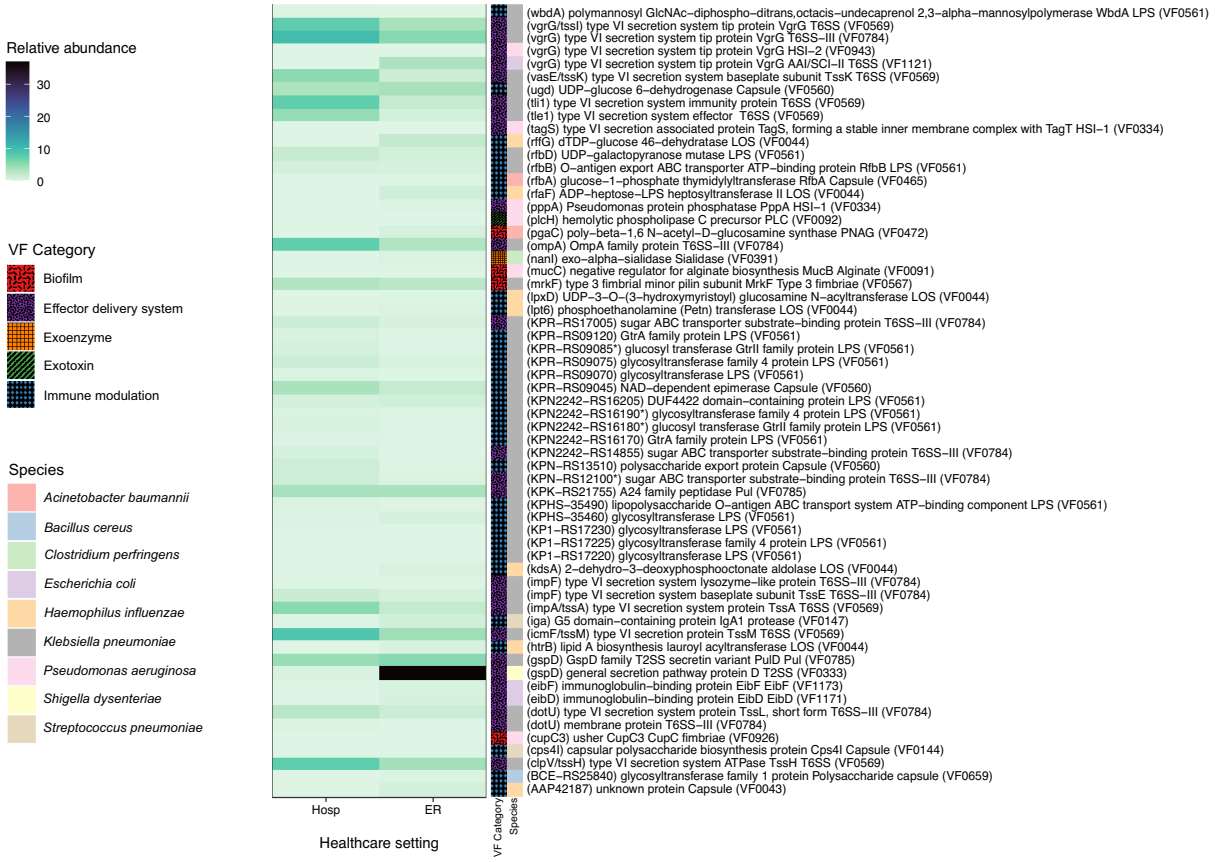

Fig. 3 Statistically significant virulence factors between the study groups were associated with nine bacterial taxa ($p < 0.05$). The figure shows two colored stripes, the first of which corresponds to the category of virulence factor identified (Biofilm, Effector delivery system, Exoenzyme, Exotoxin, and Immune modulation), and the second colored stripe represents the bacterium associated with that virulence factor

systems ($n = 18$) were more abundant in *Klebsiella pneumoniae* within the Hosp group. The most prominent *K. pneumoniae* VFs were linked to capsule polysaccharide synthesis (e.g., KPR-RS09045: NAD-dependent epimerase) and secretion systems (e.g., *vgrG*: type VI secretion system tip protein VgrG T6SS-III).

Although *E. coli* was one of the most abundant bacteria, only three VFs belonging to this species showed a differentially significant abundance between groups. Specifically, *E. coli* VFs related to secretion systems and immunoglobulin-binding proteins (*vgrG*, *eibF*, and *eibD*) were more abundant in the ER group. Additionally, the exoenzyme exo-alpha-sialidase (*nanI*) from *Clostridium perfringens* exhibited a significantly higher abundance in the ER group (0.26%, $p = 0.029$) (Fig. 3).

A detailed description of the virulence factors associated with *C. difficile* and *C. perfringens* was conducted, given their relevance as clinically important pathogens. For this analysis, only samples in which these species were observed ($n = 17$) were included. Eleven exoenzymes and seven exotoxins were identified for these species (Additional file 4: Fig. S3). However, statistical tests were not performed due to the limited sample size (Hosp = 12, ER = 5). *C. difficile* toxins were detected in three Hosp group samples, with Toxin A (*tcdA*) being the most abundant (mean: 7.33%). In contrast, *C. perfringens* toxins were found in most Hosp (10/12) and all ER (5/5) samples, with sialidases and hyaluronidases being the most prevalent (mean: 10.47%) (Additional file 3: Fig. S3).

Identification of antimicrobial resistance markers from metagenomic reads

A total of 2,875 ARM hits were identified from read-based analyses using the CARD database. Of these, 1,595 ARM hits were classified as chromosome-associated (Additional file 2: Table S5) and 1,280 as plasmid-associated (Additional file 2: Table S6), based on CARD reference annotations. This classification reflects the typical genomic context reported in the reference database and does not imply direct inference of the physical genomic location of these markers in the metagenomic data. ARMs hits were aggregated by antibiotic drug class for downstream analyses, spanning 20 distinct drug classes according to CARD classification. The mean number of chromosome-associated marker reads was 991.43 (SD: 3,357.91) in the Hosp group and 745.8 (SD: 3,117.03) in

the ER group, with no statistically significant differences observed ($p > 0.05$) (Fig. 4a). In contrast, plasmid-associated markers showed significantly higher read counts in the Hosp group (mean: 1,235.01; SD: 4,293.3) compared to the ER group (mean: 845.82; SD: 3,823.62) ($p = 0.014$) (Fig. 4b). Chromosome-associated ARMs conferring resistance to aminoglycosides, peptides, and tetracyclines were predominant in both groups (Additional file 4: Fig. S4a). Notably, significant differences were observed in chromosome-associated ARMs associated with glycopeptides ($p = 0.005$), lincosamides ($p = 0.018$), sulfonamides ($p = 0.034$), and triclosan ($p = 0.03$) (Additional file

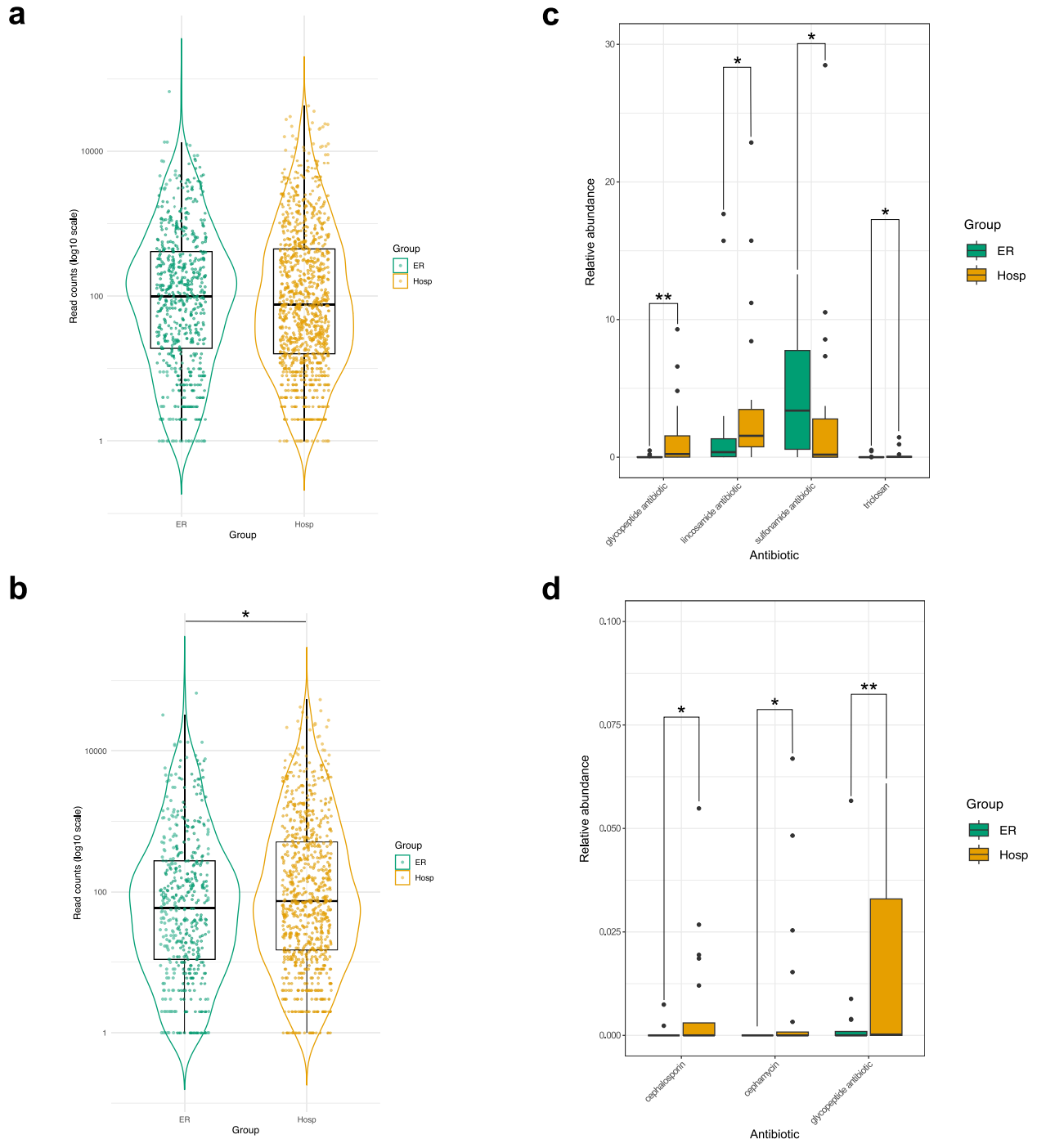

Fig. 4 Read counts of antimicrobial resistance markers (AMRs) in the hospitalized (Hosp) and emergency room (ER) groups. Boxplots show the distribution of ARM read counts across study groups on a $\log_{10}$ scale. **a.** Chromosome-associated ARMs, **b.** Plasmid-associated ARMs. **c.** Chromosome-associated ARMs were classified by antibiotic type and exhibited significant abundance differences between groups ($p < 0.05$). **d.** Plasmid-associated ARMs classified by antibiotic type and exhibiting significant differences in abundance between groups ($p < 0.05$)

4: Fig. S5a). Sulfonamide-resistance ARMs were particularly abundant in the ER group (Fig. 4c).

In the case of plasmid-associated markers, ARMs conferring resistance to aminoglycosides and tetracyclines were predominant in both groups (Additional file 4: Fig. S4b). However, these markers did not show significant differences in abundance between the groups ($p > 0.05$). In contrast, ARMs associated with resistance to cephalosporins ($p = 0.038$), cephamycins ($p = 0.028$), and glycopeptides ($p = 0.0066$) exhibited significant differences (Additional file 4: Fig. S5b). Notably, these markers showed a higher relative abundance in the Hosp group compared to the ER group (Fig. 4d).

Metagenome-assembled Genome (MAG) reconstruction

A total of 747 MAGs were reconstructed, including 441 from Hosp patient samples and 306 from ER patient samples. Most MAGs (n = 492) were classified as high-quality (HQ), while 220 were medium-quality (MQ) and 35 were low-quality (LQ) (Fig. 5a). Only HQ MAGs were used for downstream analyses, featuring an average completeness of 96.76% and contamination of 1.14%. These HQ MAGs ranged from 1.18 to 7.07 megabases in size, with a mean N50 of 77.15 kb, and an average GC content of 47.77%. Taxonomically, the HQ MAGs were assigned to 11 bacterial phyla, with Firmicutes predominating in both groups (Hosp: n = 185; ER: n = 118), followed by Bacteroidota, Actinobacteriota, and Proteobacteria (Fig. 5b). Detailed statistics and classifications of HQ MAGs are summarized in Table S7 (Additional file 4). This broad taxonomic representation reflects the complexity and diversity of the gut microbial communities in both hospital and community-onset diarrhea.

Regarding clinically relevant pathogens,18 out of 24 samples (75%) from the Hosp group and 10 out of 17 samples (58.8%) from the ER group yielded at least one MAG corresponding to a known diarrhea-associated pathogen [65, 66], suggesting a high prevalence of such pathogens in the hospitalized group.. Among these, most of the *Clostridium* spp. MAGs (including *Clostridium symbiosum*, *Clostridium saccharolyticum*_A, *Clostridium innocuum*, *Clostridium leptum*, *Clostridium scindens*, and *Clostridium* sp.) were reconstructed from Hosp samples under the applied assembly conditions. Similarly, *Klebsiella pneumoniae* and *Enterococcus faecium* were also only detected in Hosp patients. In contrast, *Escherichia coli*, *Escherichia flexneri* (*Shigella flexneri* classified as *Escherichia* in the reference database), *Enterococcus faecalis*, and *Clostridium ventriculi* MAGs were from ER samples under the applied assembly conditions. Full statistics and MAG classifications are provided in Table S7 (Additional file 4).

Functional analysis of MAGs

Functional annotation of HQ MAG using the COG (Clusters of Orthologous Groups) database revealed 21 functional categories across all genomes. As shown in Fig. 6, a substantial proportion of annotated genes were

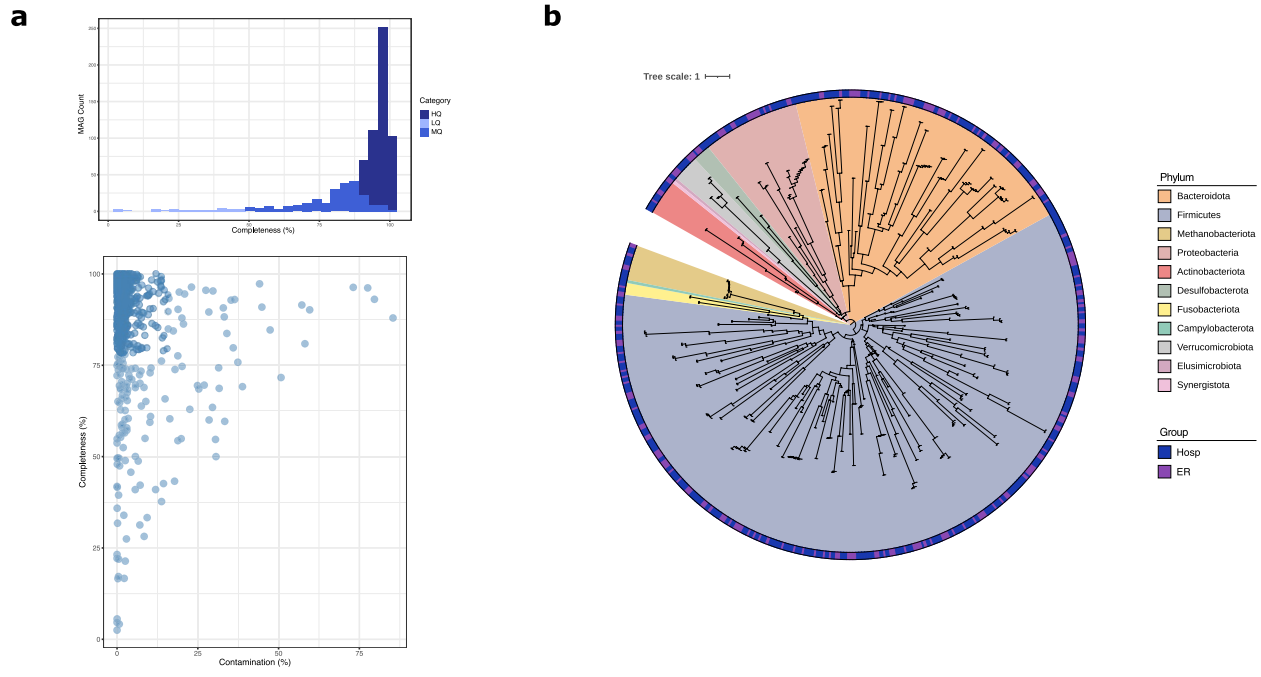

Fig. 5 Main quality statistics and taxonomic assignment of reconstructed MAGs. **a.** Barplot showing the number of reconstructed HQ, MQ, and LQ MAGs (upper panel) with their completeness and contamination estimation (lower panel). **b.** Phylogenetic reconstruction of 492 HQ MAGs from each study group

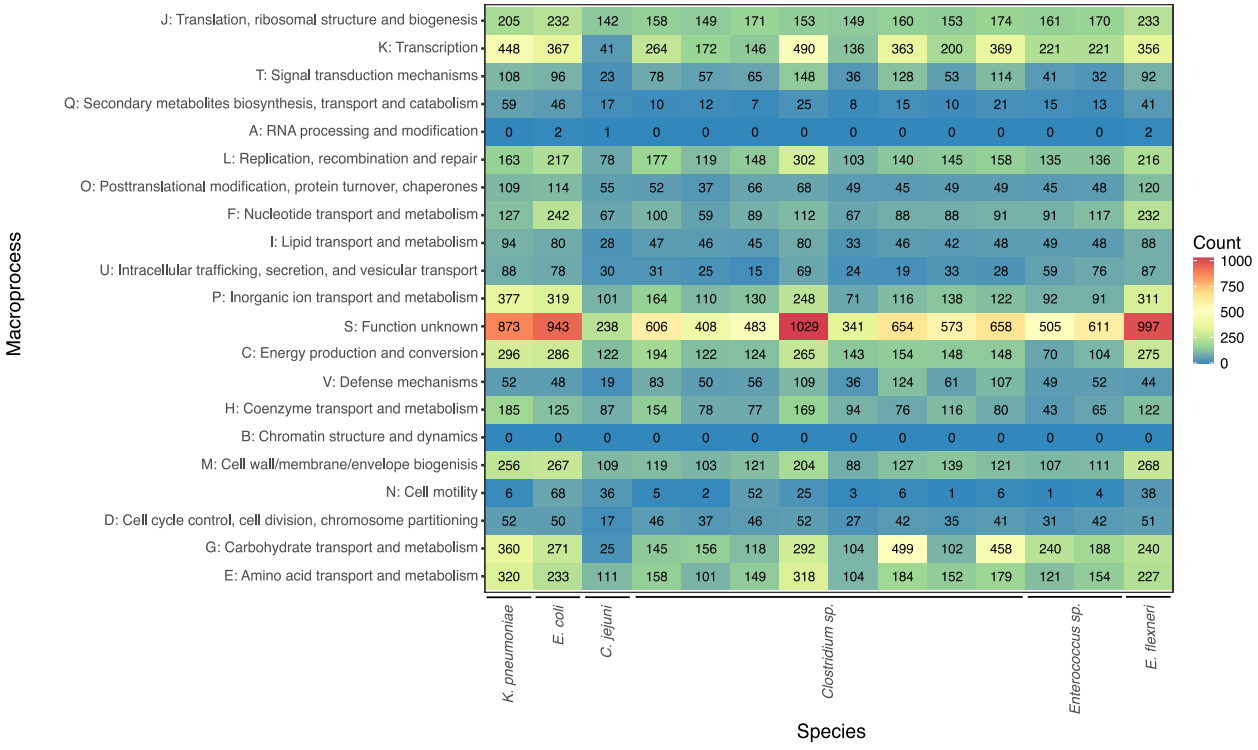

Fig. 6 Functional coding potential of reconstructed metagenome-assembled genomes (MAGs) associated with diarrhea-causing bacterial species based on Clusters of Orthologous Genes (COGs)

classified under "S: Function unknown," highlighting the persistent gap in functional characterization of microbial genes. Nonetheless, most of known functions were associated with essential cellular processes such as transcription (K), carbohydrate transport and metabolism (G), amino acid transport and metabolism (E), Energy production and conversion (C), and translation, ribosomal structure, and biogenesis (J).

The analysis of VFs also revealed a distinct distribution across species and between clinical settings. Among Hosp patients, *K. pneumoniae* MAGs were the most enriched in virulence traits, carrying multiple VFs such as *iutA* (aerobactin siderophore receptor, n = 4), *traT* (outer membrane complement resistance protein, n = 4), and *terC* (tellurium ion resistance protein, n = 4), which are associated with immune evasion and enhanced environmental survival. *E. faecium* MAGs also encoded the *acm* gene (collagen adhesin, n = 2), a known mediator of tissue adhesion and pathogenesis in enterococcal infections. Additionally, some *Clostridium* spp. MAGs harbored the *groEL* gene (molecular chaperone GroEL), although its role in virulence remains debated. In contrast, among ER samples, VFs were more prevalent in *E. coli* and *E. flexneri* MAGs. The most frequent virulence marker in both species was *gad* (n = 7 in *E. coli*; n = 6 in *E. flexneri*), encoding glutamate decarboxylase, which contributes to acid resistance and colonization. Other notable VFs included *lpfA* (long polar fimbriae subunit A), *capU* (hexosyltransferase), and *air* (adhesin involved in intestinal colonization), which collectively enhance adherence and invasion in host tissues. *E. faecalis* MAGs carried *efaAfs* (endocarditis antigen), linked to biofilm formation and persistent infections (Additional file 5: Table S8).

MAGs assigned to clinically relevant diarrhea-associated species exhibited notable differences in ARMs content. Among Hosp patients, *K. pneumoniae* MAGs harbored *oqxA* as the most frequent resistance marker (n = 6), encoding a subunit of the RND-type efflux pump OqxAB, known to confer resistance to fluoroquinolones and other antimicrobials. *E. faecium* MAGs, frequently carried *aac(6')-Ii* (n = 3), an aminoglycoside acetyltransferase associated with resistance to gentamicin and related drugs. Additionally, *Clostridium* spp. MAGs (including *C. symbiosum*, *C. innocuum*, and *C. leptum*) showed limited resistance content, with *tet(W)* (n = 2) as the most common ARM, a ribosomal protection protein conferring tetracycline resistance. In contrast, MAGs from ER group exhibited heterogeneous resistance patterns. *E. coli* and *E. flexneri* MAGs carried a high number of ARMs, with *aph(6)-Id* as the most frequent gene in both species (n = 7 and n = 6, respectively). This gene encodes an aminoglycoside phosphotransferase that mediates streptomycin resistance, suggesting a

widespread aminoglycoside resistance phenotype among these enteric pathogens. *E. faecalis* MAGs, also exclusive to ER patients, frequently contained *lsa(A)* (n = 2), an ABC-F family ribosomal protection protein implicated in lincosamide resistance (Additional file 5: Table S9).

Discussion

The taxonomic composition of the gut microbiome can be affected when there is a disruption in homeostasis, especially in a scenario of diarrhea, which may be accompanied by a significant increase in bacteria belonging to the phylum Proteobacteria [67, 68]. The above coincides with the findings of our study, in particular with the high abundance of the Enterobacteriaceae family in both study groups (Additional file 3: Fig. S2c) [6]. Pathogenic members of this bacterial family, such as *K. pneumoniae* and *E. coli*, were notably abundant in the study groups (Fig. 1c); however, their presence in the microbiome does not indicate infection or the cause of these patients' diarrhea. Although commonly found in the gut microbiome, these bacteria are essential in public health as they are frequent agents of nosocomial infections [69, 70]. Future studies could focus on strain-level profiling of the circulating species within hospital settings, which may enhance pathogen surveillance and contribute to the prevention of nosocomial infections.

Phages account for 97.7% of the gut virome. Although they are still an object of study, these phages may influence the abundance of other microbiome members, play various roles in the gut microbiome, and have their signatures modified in the setting of a particular disease [71, 72]. Our results showed a high abundance of Myoviridae, Podoviridae, and Siphoviridae in both study groups (Additional file 3: Fig. S2d), consistent with previous studies indicating a predominance of these viral families in healthy and diseased individuals [73, 74]. Further, our results show that the two study groups were not differentiated by bacterial species but by viral species. Here, crAss-like phages and uncultured *crAssphages* presented a high abundance in patients of both study groups (Fig. 1d). This result is concordant with recent studies describing these viruses as the most abundant and possibly one of the relatively stable members of the human gut microbiome over time [74–76].

The study of variations in phage composition has been investigated in diseases such as inflammatory bowel disease (IBD), colorectal cancer, and type 2 diabetes, where an increase in the abundance of particular phages has been reported and which has even suggested that alterations in phage communities may be disease-specific [77–79]. Our observations show a contrasting trend in the Hosp group, where there was a decrease in the abundance of *K. pneumoniae*- and *Escherichia*-specific phages (Fig. 2). The low abundance of these phages may be attributed to several factors, the most significant being the induction of prophages, commonly seen in gut homeostasis imbalance or during antibiotic administration [80].

These potential dynamics between phages and bacteria are part of a snapshot of the patient's microbiome at the time of sample collection. Hence, the interactions between the phages and their hosts may change over time according to the different co-evolutionary models described for phages and bacteria [81–83]. A limitation of this study is that phage abundances were not normalized to the abundance of their predicted bacterial hosts. As a result, differences in phage detection may partly reflect variation in host bacterial abundance, genome copy number, or phage life cycle dynamics rather than true phage depletion. Nonetheless, ANCOM-BC detected phages as differentially abundant, but not their corresponding bacterial hosts, which suggests that phage depletion may not be solely driven by host abundance differences. Future work integrating host-normalized phage abundance or phage-host ratio is essential to provide insight into the dynamics of these phages with their bacterial host in diarrhea scenarios over time. Additionally, factors that can modify the composition and interaction between phages and bacteria, such as nutrient availability, metabolites, bacterial and viral viability, and patient-related factors (e.g., health status, treatments), should be considered [80].

The analysis of virulence factors in the groups showed a high abundance of *K. pneumoniae* VFs in the Hosp group, in particular, the gene involved in the synthesis of capsule polysaccharides (*KPR-RS09045:* NAD-dependent epimerase) (Fig. 3). Several reports note the importance of the capsule of *K. pneumoniae* in evading the immune system response during infection and hindering the antibacterial action of antimicrobial peptides [84, 85]. For the same bacterium, we also observed genes encoding for Type VI Secretion System (T6SS) VgrG proteins (Fig. 3), which play a crucial role in the interspecies competition of *K. pneumoniae*. Additionally, it has been described that the T6SS in *K. pneumoniae* can mediate intraspecies competition, adhesion, and invasion and even mediate the acquisition of resistance genes [86, 87].

Our results on the presence of *C. difficile* virulence factors only in samples belonging to the Hosp group (Additional file 3: Fig. S3) are concordant with the prevalence of this bacterium in health-care facilities [88–90]. Similar to our results, the study by Herrera et al. (2022) reported the presence of *C. difficile* toxins A and B in hospitalized patients, as opposed to patients who acquired *C. difficile* infection in the community [34]. Forero et al. (2019) reported the frequency of *C. perfringens* infection in patients from two hospital centers in Bogotá, Colombia, where they found a high frequency of *C. perfringens*

infection in patients with community-acquired diarrhea compared to those who acquired it in a healthcare facility [91]. Likewise, our results indicate the presence of *C. perfringens* toxins in samples from both study groups. The above highlights the possible circulation of different *C. perfringens* toxins within the community, especially considering the relatively short sampling period for ER patients (48 to 72 h). Within this repertoire of genes coding for *C. perfringens* toxins, *nanI* sialidase (Fig. 3; Additional file 3: Fig. S3) is noteworthy, as it constitutes the highest exosialidase activity at the in vitro level of the bacterium and may even enhance the role of other toxins such as CPE, ε-toxin, and β-toxin [92–94]. Although *C. difficile* and *C. perfringens* were detected through metagenomic sequencing, the absence of confirmatory tests such as PCR or culture represents a limitation of this study. Likewise, considering the modest sample size, these findings should be interpreted as exploratory and hypothesis-generating rather than inferential. Nevertheless, the identification of virulence factors associated with these species provides valuable insight into their potential presence and highlights the utility of metagenomics in pathogen surveillance.

Regarding the identified ARMs circulating in the microbiome, we highlight the high number of plasmid-associated markers in the Hosp group (Fig. 4b; Additional file 3: Fig. S4b). Currently, ARMs are known to be ubiquitous among pathogenic and non-pathogenic bacteria. Likewise, they can be found in animals, water, and the environment and thus, can be transmitted between and within these reservoirs [95–97]. Although the routes of ARM acquisition are so broad, within hospitalization, different factors (e.g., constant antibiotic administration, acquisition of resistant organisms found in the hospital environment, horizontal gene transfer, among others) can contribute to the acquisition of particular ARMs in this patient population, and even to the proliferation of resistant bacterial species [98, 99]. In the case of ARMs identified in ER patients, it is equally difficult to track the routes of acquisition of these markers, as it is necessary to consider additional factors, such as the consumption of antibiotics before admission to the hospital. It is valuable to highlight that the presence of the ARMs reported here in the patients of both groups does not indicate a phenotype of resistance to these antibiotics. Therefore, future studies could integrate phenotypic tests to confirm patients' resistance to specific antibiotics, enabling the appropriate treatment administration to the patients.

MAGs were reconstructed to obtain a detailed description of key microbial taxa of the gut microbiome of patients in both clinical settings. A total of 492 high-quality MAGs, including representative genomes for several diarrhea-associated species, underscores the utility of metagenomics as a robust approach for unraveling microbiome dynamics, facilitating the simultaneous recovery of multiple species' genomes without the need for culturing, and offering valuable insights into the microbiome composition, functional potential, and pathogen diversity. The broad taxonomic representation spans 11 bacterial phyla, with Firmicutes being dominant in both the Hosp and ER groups, a pattern consistent with prior human gut studies. Our data revealed that 75% of Hosp samples and 58.8% of ER samples contained at least one MAG corresponding to a known diarrheagenic pathogen. MAGs corresponding to *K. pneumoniae* and *Clostridium* spp. were recovered from Hosp patients under the applied assembly framework, whereas MAGs classified as *E. coli*, *E. flexneri*, *E. faecalis*, and *C. ventriculi* were recovered from ER patients. However, MAG-based presence/absence patterns should be interpreted with caution, as genome recovery depends on sequencing depth, coverage, and the performance of assembly and binning algorithms. Low-abundance organisms or highly diverse strain populations may be fragmented or not recovered, even when present in the community. Consequently, the absence of a given MAG should not be interpreted as definitive biological absence, but rather as non-recovery under the applied sequencing and assembly methodology. Despite these limitations, our findings provide a high-resolution snapshot of pathogen presence across settings, offering a valuable baseline for future surveillance and epidemiological studies, accordingly with recent works that have further demonstrated MAG reconstruction's value for mapping gut-associated opportunistic pathogens (Gupta & Almeida, 2025).

Functional annotation of high-quality MAGs provided detailed insights into the metabolic and resistance capacities of gut-associated pathogens in both clinical settings. Most annotated COGs corresponded to genes essential for core cellular function, such as transcription, carbohydrate and amino acid metabolism, and nucleotide biosynthesis (Fig. 6), consistent with the metabolic centrality of the gut microbiome previously described in human and animal studies (Fujisaka, et. al., 2023). These functions are fundamental for microbial survival, host adaptation, and persistence, and their enrichment underscores the conserved metabolic strategies of enteric bacteria in both hospital- and community-onset diarrhea. Notably, genes involved in nucleotide metabolism (which influence both virulence and antibiotic susceptibility) were predominant across taxa, consistent with previous reports linking nucleotide flux to antimicrobial efficacy and stress response mechanisms [100–102].

Analysis of obtained MAGs revealed that many of the most frequent ARMs, such as *oqxA* in *K. pneumoniae* and *aph (6) -Id* in *E. coli* and *E. flexneri*, encode proteins involved in efflux-mediated resistance, as classified by the CARD database [45] (Additional file 5, Table S9).

The carriage of the efflux genes in the bacterial chromosome gives bacteria the advantage of expressing them for survival under stress conditions induced by a particular antibiotic. This system can also confer resistance to more than one class of antibiotic and even to disinfectants and dyes, as observed in the *E. coli* MAGs [103, 104]. Species- and setting-specific differences in ARMs across MAGs reflected ecological adaptation to clinical environments. In the Hosp group, the predominance of *K. pneumoniae* and *E. faecium* carrying efflux and aminoglycoside resistance genes (e.g., *oqxA*, *aac(6')-Ii*) likely reflects selective pressures from hospital antibiotic regimens. In contrast, *Clostridium* spp. exhibited minimal resistance potential, consistent with limited horizontal gene transfer in anaerobic niches. MAGs from ER patients showed broader ARM diversity, especially among *E. coli*, *E. flexneri*, and *E. faecalis*, aligning with previous evidence of community-level reservoirs of clinically relevant resistance markers (Li et al., 2022). These findings underscore the value of genome-resolved metagenomics for contextualizing resistance dynamics in gut pathogens. However, it is important to acknowledge that gene presence does not imply expression; future integration of (meta)transcriptomic approaches could provide more accurate insights into active resistance mechanisms and guide targeted treatment decisions to minimize the risk of treatment failure.

In the present study, we employed shotgun metagenomics to characterize the gut microbiome of a specific population of patients with diarrhea at Hospital Universitario Mayor–Méderi following a read-based and an assembly-based approach. It is important to note that the Hosp and ER departments of Hospital Universitario Mayor–Méderi treat a diverse population in terms of age, sex, and disease. Therefore, the only common criterion for patients recruited for this study was the presence of diarrhea. Our key results point to the close relationship that may exist between phages and bacteria in the gut microbiome of these patients (Fig. 2; Additional file 3: Fig. S6). Additionally, we observed a high prevalence of VFs from pathogenic bacteria such as *K. pneumoniae*, *C. difficile*, and *C. perfringens* in the study groups (Fig. 3; Additional file 3: Fig. S3), along with a high number of plasmid-associated ARMs in the Hosp group (Fig. 4b; Additional file 4: Fig. S4b). In particular, the reconstruction of genomes from the metagenomic reads (MAGs) was advantageous in gaining insight into the possible dynamics and interactions of key members of the gut microbiome.

While our study offers valuable insights and is among the few to describe the gut microbiome of Colombian patients using both read- and assembly-based approaches, it has limitations. These include the relatively small number of enrolled patients and the limited metadata collected for each individual, which restricted the scope of downstream analyses. In particular, emergency room patients were enrolled based solely on the presence of diarrhea at presentation, and detailed information regarding the specific reasons for emergency consultation, recent treatments, dietary intake, or other short-term clinical factors was not systematically collected; therefore, the potential influence of these variables on microbiome composition cannot be fully excluded. In addition, the absence of a healthy control group precludes direct inference of microbiome disruption relative to a baseline healthy state; therefore, our findings are interpreted in terms of distinct microbiome features associated with community-onset and hospital-onset diarrhea rather than definitive dysbiosis. An imbalance in sex distribution was also observed in the ER group. Although sex-related differences in gut microbiome composition have been reported in certain contexts, the modest sample size did not allow for robust sex-stratified analyses in this study, and potential sex-associated effects on microbiome composition cannot be fully excluded. Future studies with larger and more balanced cohorts will be required to develop a more comprehensive description of the gut microbiome in this patient population, including the evaluation of sex-specific effects on microbiome composition and function. Such studies would benefit from integrating multi-omics approaches (e.g., metatranscriptomics, metabolomics, and single-cell analyses) with complementary phenotypic and clinical data. Likewise, these future studies could be advantageous for profiling eukaryotic communities, which could not be addressed in the present study, and play a crucial role in the gut microbiome. However, this study contributes to the existing knowledge about alterations in the gut microbiome in patients from Hosp and ER departments of a healthcare facility in Bogotá, Colombia. The VFs and ARMs identified in this study may serve as candidate genetic targets for the development of rapid molecular diagnostics, such as PCR- or isothermal amplification-based assays, for early detection of multidrug-resistant or hypervirulent pathogens in clinical settings. While further validation in larger and independent cohorts is required, this study demonstrates the utility of integrating read-based and genome-resolved metagenomic approaches to characterize microbiome features associated with community-onset and hospital-onset diarrhea. Although exploratory, the taxonomic and functional profiles described here provide a foundation for the development of targeted diagnostic and surveillance strategies, support tailored infection control interventions, and contribute to improving patient care protocols by enabling the early identification of potential reservoirs of opportunistic pathogens, ARMs, and VFs.

Abbreviations

Hosp	Hospitalized group
ER	Emergency room group
VFs	Virulence factors
ARMs	Antibiotic resistance markers
MAGs	Metagenome-assembled genomes
ANCOM-BC	Analysis of compositions of microbiomes with bias correction
RGI	Resistance Gene Identifier
ANIb	Average nucleotide identity using blast
COGs	Cluster of orthologous genes
SD	Standard deviation
LFC	Log fold change
MLST	Multilocus sequence typing
IBD	Inflammatory bowel disease

Supplementary Information

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Additional file 1

Additional file 2

Additional file 3

Additional file 4

Additional file 5

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Author contributions

LV processed fecal samples, data analyses, and visualization, and wrote the original draft. CB defined the methodological approach, acquired funding, administered the project, and reviewed and edited the manuscript. DD, CL, MCM-M, and CP recruited patients, collected patient data, and reviewed and edited the manuscript. AA, DP, GR-L, and ES reviewed and edited the manuscript. AS stored and organized the fecal samples and revised and edited the manuscript. GH developed conceptualization, supported data analyses, and reviewed and edited the manuscript. JDR developed conceptualization, defined the methodological approach, acquired funding, supervised the project, and reviewed and edited the manuscript. MM developed conceptualization, defined the methodological approach, acquired funding, administered the project, and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Data availability

The dataset(s) supporting the conclusions of this article is(are) available in the European Nucleotide Archive (ENA) repository under accession number PRJEB77410 (https://www.ebi.ac.uk/ena/browser/view/PRJEB77410).

Declarations

Ethics approval and consent to participate

The current project was conducted with the approval of the Research Ethics Committee of the Universidad del Rosario (Approval code DVO005 1856-CV1493). This study was considered low risk according to Resolution 8430 of 1993 of the Ministry of Health of Colombia.

Competing interests

The authors declare no competing interests.

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