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Exploring the genomic traits of infant-associated microbiota members from a Zimbabwean cohort

Taona Emmah Mudhluli1,2†, Magdalena Kujawska3†, Julia Mueller3, Angela Felsl3, Bastian-Alexander Truppel3,4, Lindsay J. Hall3,5,6,7, Inam Chitsike8, Exnevia Gomo1 and Danai Tavonga Zhou1

Abstract

Introduction  Our understanding of particular gut microbiota members such as Bifidobacterium and Enterococcus in low-middle-income countries remains very limited, particularly early life strain-level beneficial traits. This study addresses this gap by exploring a collection of bacterial strains isolated from the gut of Zimbabwean infants; comparing their genomic characteristics with strains isolated from infants across North America, Europe, and other regions of Africa.

Materials and method  From 110 infant stool samples collected in Harare, Zimbabwe, 20 randomly selected samples were used to isolate dominant early-life gut microbiota members Bifidobacterium and Enterococcus. Isolated strains were subjected to whole genome sequencing and bioinformatics analysis including functional annotation of carbohydrates, human milk oligosaccharide (HMO) and protein degradation genes and clusters, and the presence of antibiotic resistance genes (ARGs).

Results  The study observed some location-based clustering within the main five identified taxonomic groups. Furthermore, there were varying and overall species-specific numbers of genes belonging to different GH families encoded within the analysed dataset. Additionally, distinct strain- and species-specific variances were identified in the potential of Bifidobacterium for metabolizing HMOs. Analysis of putative protease activity indicated a consistent presence of gamma-glutamyl hydrolases in Bifidobacterium, while Enterococcus genomes exhibited a high abundance of aspartyl peptidases. Both genera harboured resistance genes against multiple classes of antimicrobial drugs, with Enterococcus genomes containing a higher number of ARGs compared to Bifidobacterium, on average.

Conclusion  This study identified promising probiotic strains within Zimbabwean isolates, offering the potential for early-life diet and microbial therapies. However, the presence of antibiotic resistance genes in infant-associated microbes raises concerns for infection risk and next-stage probiotic development. Further investigation in larger cohorts, particularly in regions with limited existing data on antibiotic and probiotic use, is crucial to validate these initial insights.
**Impact statement** This research represents the first investigation of its kind in the Zimbabwean context, focusing on potential probiotic strains within the early-life gut microbiota. By identifying local probiotic strains, this research can contribute to the development of probiotic interventions that are tailored to the Zimbabwean population, which can help address local health challenges and promote better health outcomes for infants. Another essential aspect of the study is the investigation of antimicrobial resistance genes present in Zimbabwean bacterial strains. Antimicrobial resistance is a significant global health concern, and understanding the prevalence and distribution of resistance genes in different regions can help inform public health policies and interventions.

**Introduction**

Commendable efforts are being made by individual researchers and groups in Zimbabwe to analyse the human gut microbiota, utilizing ongoing cohorts [1]. Recent research includes studies on gut microbiome signatures associated with colorectal cancer in adults [2], the composition of the intestinal microbiota in pregnant women [3], and HIV-infected infants on cotrimoxazole [4]. However, studies exploring strain level diversity of *Bifidobacterium* and other key microbes like *Enterococcus*, and their role in health during infancy, lag in Zimbabwe and other low and medium-income countries (LMICs) [1].

Breastfeeding plays a crucial role in shaping the gut microbiota of infants, and the presence of multiple *Bifidobacterium* communities is a significant feature of the early-life developmental window [5–7]. Specific probiotic strains of *Bifidobacterium* and *Enterococcus*, as well as other genera such as *Lactobacillus*, contribute to health benefits, including enhanced immune function, improved digestion, and reduced risk of allergies and infections by supporting the development of a healthy gut microbiome [8]. Probiotics are defined by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [9].

Breast milk contains a variety of human milk oligosaccharides (HMOs), which are complex sugars that cannot be metabolized by infants [6]. Instead, these HMOs play a crucial role in establishing an infant-specific gut microbiota, with bifidobacterial species such as *Bifidobacterium longum* subsp. *infantis* and *Bifidobacterium breve* being particularly abundant [6]. Studies have shown that bifidobacteria possess a diverse array of carbohydrate-active enzymes (CAZymes) that enable them to degrade and utilize host- and diet-derived complex carbohydrates such as HMOs and plant polysaccharides, including pectins and resistant starch [10]. Similarly, *Enterococcus* species have been found to harbour genes encoding various glycoside hydrolases, polysaccharide lyases, and carbohydrate esterases, indicating their capacity to metabolize a broad spectrum of carbohydrates [11, 12]. Studies on milk carbohydrate metabolism pathways and HMO utilization by *Bifidobacterium* or other intestinal bacteria, from LMICs, including Zimbabwe, have not been reported to date [1].

There is a concern that microbes may harbour antibiotic resistance genes (ARGs), with a potential of transfer to pathogenic gut bacteria [13, 14]. This could potentially contribute to the spread of antimicrobial resistance, which poses an important problem in LMICs, where infants are particularly vulnerable to intestinal infections. Enterococcal species are typically found in the gut, bowel, throat, mouth, and vagina as commensals, with *Enterococcus faecalis* and *Enterococcus faecium* considered to be potential opportunistic pathogens [15]. Strains of these species can harbour virulence genes that have been associated with infections in humans, including urinary tract and sepsis [13, 15]. The diversity and abundance of these genes can vary across different populations due to differences in diet, ethnicity, environmental exposures, and genetic factors [16]. Understanding these geographical variations is crucial for unravelling the complex interactions between the gut microbiota, and early-life health or disease.

This study investigates the genomic makeup of *Bifidobacterium* and *Enterococcus* strains isolated from Zimbabwean infants with a focus on their potential for carbohydrate metabolism and the presence of antibiotic-resistance genes. These findings offer valuable insights that can potentially contribute to the development of early-life dietary microbial therapies. The data obtained from this study were compared with data from North America, Europe, and other African countries.

**Materials and methods**

**Study participants**

The research adhered to the guidelines outlined in the Declaration of Helsinki, where the participants’ mothers were adequately informed about the study before providing their written consent for their infants to participate. Additionally, the study was initiated following ethical approval from multiple committees, including the Harare City Council Ethics Committee (3/7), the Joint Research Ethics Committee for the University of Zimbabwe Faculty
of Medicine and Health Science and Parirenyatwa Group of Hospitals (353/2021), Medical Research Council in Zimbabwe (MR CZ A/2832), and the Research Council of Zimbabwe reference number 04694. A total of 110 stool samples were collected from 110 infants who visited six different municipal clinics for their routine 6-week “baby clinic” check-up. Collected stool samples were promptly stored in a freezer set at -80°C until transferred under cold-chain conditions to the Chair of Intestinal Microbiome, Technical University of Munich, Germany for processing.

Isolation and preliminary identification of recovered isolates

From a total of 110 infant stool samples, we selected a random set of 20. These infants were 6 weeks old, vaginally delivered, not on antibiotics and exclusively breastfeeding. However, the history of the mothers’ antibiotics use was not known. We performed bacterial isolations targeting potentially beneficial early-life microbiota members; ~ 100ug of stool was added to 1ml total Phosphate-Buffered Saline (Sigma-Aldrich, USA) and diluted to a factor of 10^{-3}. We streaked 100ul of diluted stool on Reinforced Clostridial Medium (RCM) agar (Oxoid, UK) supplemented with cysteine-HCL (0.5g/L) (Sigma-Aldrich, USA). After anaerobic incubation for 48 h in an anaerobic cabinet (90% Nitrogen, 5% Hydrogen, 5% Carbon Dioxide) (Baker Ruskinn, UK), selected colonies were transferred from RCM to de Man, Rugosa, and Sharpe (MRS) agar (BD Difco™, USA) supplemented with mupirocin (0.05g/L) (Sigma-Aldrich, USA) and cysteine-HCL (0.5g/L) and cultivated at 37°C in an anaerobic environment for 48h. Colonies were re-streaked to purity on MRS plates. A single colony was then picked from the final re-streak, placed in 20ml of MRS broth with mupirocin (0.05g/L) and cysteine-HCL (0.5g/L), and cultivated for 48h under anaerobic conditions at 37°C. Thereafter, the culture was split into two 10ml subcultures and centrifuged for 15 min, and the supernatant was discarded. One pellet was stored at -20°C until DNA extraction. The other pellet was then resuspended in 1ml 20% glycerol and RCM media aliquoted into cryovials and stored at -80°C.

DNA was extracted from pure bacterial cultures using the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) after lysis in the FastPrep-24 instrument at 6.0 speed m/s for 40s. (MP Biomedicals, USA). Partial 16S rRNA gene sequencing was performed using primers 5’-AGA GTT TGA TCC TGG CTC AG-3’; 5’-AGA GTT TGA TCA TGG CTC AG-3’ and 5’-ACG GTT ACC TGG TTA CGA CTT-3’ at Eurofins Genomics, Ebersberg, Germany. Preliminary identification of the isolates was performed with SINA aligner (v.1.2.12) [17].

Whole genome sequencing and generation of the final dataset

Based on the results of the preliminary identification, we selected Bifidobacterium and Enterococcus isolates and subjected them to whole genome sequencing on Illumina NextSeq2000 platform at the Quadram Institute Bioscience (Norwich, UK). Obtained sequencing reads were pre-processed with fastp v.0.23.2 [18]. Unicycler v.0.4.9 [19] with the “—mode conservative” option was used to produce assemblies, after which contigs below 1000bp were filtered out. We estimated genome completeness and contamination using CheckM v.1.2.0. [20], and retained sequences with completeness >99% and contamination <1%.

Additionally, the NCBI database was searched for genomes or metagenome-assembled genomes (MAGs) of infant-associated Bifidobacterium and Enterococcus strains from various geographical locations. Initially, we searched for the relevant data from African infants up to 6 months of age. However, due to limited availability of such datasets, and often incomplete infant host age metadata, we dropped the age criterion, and expanded our searches to include sequencing data from Europe and North America – which would allow comparisons of bacterial genomic features between LMICs and high-income countries. To strike a compromise between data availability and quality, we pre-filtered the downloaded publicly available data based on the CheckM completeness >95% and contamination <5%.

Next, GTDB-Tk v.2.1.0 [21] was used to classify all genomic sequences to the strain level, and python3 module pyANI v.0.2.10 with default settings was used to calculate the average nucleotide identity values (ANI) [22]. Species delineation cut-off was set at 95% identity [23]. Sequences showing identity values above 99.9% were considered identical and were removed from further analysis [24].

After such processing, the final dataset comprised of 81 unduplicated genomes from Zimbabwe [16], Mozambique [17], Tanzania [6], USA [10], UK [25], and 6 type strain genomes representative of infant-associated Bifidobacterium and Enterococcus species—Bifidobacterium longum subsp. infantis, Bifidobacterium longum subsp. longum, Bifidobacterium breve, Bifidobacterium pseudocatenulatum, Enterococcus faecium and Enterococcus faecalis (Supplementary Table 1). All genomes were annotated using Prokka v.1.14.6 [26].

Genomic analysis

Mashtree v.1.2.0 [27] with default settings was used to assess the relatedness of strains included in the analysis. Genomic functional traits were profiled using
eggNOG-mapper v.2.1.5 [28] with eggNOG database v.5.0 [29] and METABOLIC v.4.0 [30]. Principal component analysis was performed using factoextra v.1.0.7 [25] package in R v.4.2.3 [24]. Prediction of the presence of HMO clusters in *Bifidobacterium* was performed by comparing genomes included in the final dataset to known bifidobacterial protein sequences using local blastp (e-value < 1e$^{-50}$, percentage identity > 70%). HMO clusters were annotated ‘present’ if all cluster components were identified at the above homology level. Incomplete clusters (more than three locally clustered genes) were annotated as ‘partially present’. The presence of putative antibiotic resistance genes was assessed using the CARD database, with amino acid identity > 95% considered significant (v.3.2.7) [31, 32]. R v.4.2.3 and ITOL v.6.8.1 [33] were used for data visualisation.

**Results**

**Genomic features**

In this study, we recovered and sequenced 15 bacterial isolates from stool samples of Zimbabwean infants, representative of *Bifidobacterium* and *Enterococcus* species commonly identified as members of early-life microbiota. Sequencing and assembly using the Prokka pipeline resulted in sets of contigs ranging from 16 to 178 per strain (Supplementary Table 1). The G+C content ranged from 37.29% to 37.93% for *Enterococcus* strains and 56.30% to 59.93% for *Bifidobacterium* strains, while the number of predicted coding sequences (CDS) was lower in bifidobacteria compared to enterococci, with an average of 2,084 and 2,680, respectively. Consistent with the number of predicted coding sequences (CDS) in *Bifidobacterium* genome, 51 GH components were identified at the above homology level. Incomplete clusters (more than three locally clustered genes) were annotated ‘partially present’. The presence of putative antibiotic resistance genes was assessed using the CARD database, with amino acid identity > 95% considered significant (v.3.2.7) [31, 32]. R v.4.2.3 and ITOL v.6.8.1 [33] were used for data visualisation.

**Functional annotation of infant-associated genomes—carbohydrate utilisation and protein degradation**

To take a global view of functional modules and their link to genomic diversity, the infant-associated genomes were functionally annotated and classified into COG categories against the eggNOG database. On average, 83% of CDS were assigned COG categories, with categories related to broadly defined metabolism (COG categories C, E, F, G, H, I, P, Q) representing the majority of classified CDS (35.6% on average for all analysed genomes, with 36.6% and 34.0% for *Bifidobacterium* and *Enterococcus*, respectively). Information storage and processing (COG categories A, B, J, K, L, X) was the second most abundant broad category, with an average of 24.9% CDS, while 17.6% CDS on average were assigned categories related to cellular processes and signalling (COG categories D, M, N, O, T, U, V, W, Y, Z). Poorly characterised CDS (COG categories R and S) constituted 19.1% in *Bifidobacterium* and 25.9% in *Enterococcus* genomes, on average. Within the metabolism group, CDS assigned COG categories related to carbohydrate metabolism (on average 9.50% of classified CDS, with 9.42% *Bifidobacterium* and 9.60% *Enterococcus*) and amino acid metabolism (7.72% on average, with 8.54% in *Bifidobacterium* and 6.52% in *Enterococcus*) were the most abundant in both bacterial genera (Fig. 2, Supplementary Table S4).

Inter- and intraspecies differences in gene content related to carbohydrate and amino acid metabolism have previously been described for infant-associated bacterial taxa [35–38]. We therefore assessed and compared carbohydrate-active enzyme (CAZymes) and MEROPS family repertoires of bifidobacterial and enterococcal genomes (Fig. 2, Supplementary Table S5 and S6). On average, 23 different glycoside hydrolase (GH) and polysaccharide lyase (PL) families were found in *Bifidobacterium* genomes; with an average of 56 GH genes (3.07% of CDS) in *B. pseudocatenulatum* strains, followed by 52 GH genes (2.59% of CDS) per *B. longum* genome, 51 GH genes (2.58% of CDS) per *B. breve* genome, and finally 44 GH genes (2% of CDS) per *B. infantis* genome, consistent with previous reports [6, 39].

The predominant GH in all *Bifidobacterium* genomes was GH13, which represents enzymes capable of hydrolysing alpha-1,4-glycosidic linkages in starch and similar
substrates [40], while the second most abundant GH family present in *B. longum* and *B. pseudocatenulatum*, but not in *B. infantis* and *B. breve* was GH43, which contains enzymes involved in the digestion of various aryl glycosides (Supplementary Table S5) [41]. Other abundant GH families identified across bifidobacterial genomes were GH3, GH42, and GH2, which include enzymes that hydrolyse a wide variety of glycans present in plant cell walls, with members of GH2 and GH42 families also capable of metabolising lactose, a disaccharide abundantly present in human milk. The identification of putative GH33 exo-sialidases in the genomes of *B. infantis* and *B. breve* indicates that these strains may be capable of direct utilisation of host glycans and free sialic acid metabolism [42] (Supplementary Table S5).

In enterococci, an average of 25 different CAZymes families were identified, with an average of 53 genes in *E. faecalis* (1.93% of CDS) and 51 genes (2% of CDS) in *E. faecium* genomes. Genes identified as belonging to the GH1 family, which represent enzymes that exhibit

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**Fig. 1** Relatedness of isolates in the study
β-glucosidase and β-galactosidase activity were the most abundant. Consistent with previous findings, other highly represented GH families were GH73 containing enzymes with suggested peptidoglycan hydrolase activity [43, 44], GH13 and GH109, whose members have been identified to release GalNAc from blood type A-antigen and employ an NAD$^+$-dependent hydrolytic mechanism (Supplementary Table S5) [45].

In terms of putative protease activity, *Bifidobacterium* genomes were found to contain 36 different MEROPS families on average; with an average of 40 genes in both *B. longum* subspecies (1.96 and 1.82% of CDS in *B. longum* and *B. infantis*, respectively), followed by 37 genes (1.88% of CDS) per *B. breve* genome and 33 genes (1.80% of CDS) per *B. pseudocatenulatum* genome. The predominant and consistently represented MEROPS family in bifidobacteria (range 6–7 genes) was C26, containing gamma-glutamyl hydrolases, whose human versions have previously been suggested to act as endopeptidases on gamma-linked glutamate bonds of gamma-polyglutamate substrates such as folates and anti-folates (Supplementary Table S6) [46].

*Enterococcus* genomes contained 55 different MEROPS families on average, with an average of 60 genes assigned in *E. faecalis* (2.21% of CDS) and 48 genes (1.86% of CDS) in *E. faecium*. The most represented families were M38 containing aspartyl peptidases previously implicated in antimicrobial resistance and virulence in enterococci [47], followed by M20A, C26 and S26A (Supplementary Table S6). Family M20 represents metallopeptidases, e.g. glutamate carboxypeptidases, capable of cleaving gamma-linked glutamate bonds, similar to enzymes belonging to C26, while peptidases belonging to S26A have been in the processing of newly-synthesized secreted proteins through the removal of hydrophobic, N-terminal signal peptides as the proteins are translocated across membranes [48].

**Functional annotation of *Bifidobacterium* genomes – potential for human milk oligosaccharide (HMO) utilisation**

Strains of several early life-associated *Bifidobacterium* species, namely *B. breve*, *B. infantis*, *B. longum*, and *B. pseudocatenulatum*, have been shown to contain gene clusters characterised by the presence of specific carbohydrate-active enzymes that target HMOs for degradation and metabolism [49–52]. We therefore searched for known bifidobacterial HMO gene clusters in our genomic dataset. This analysis revealed strain- and species-specific differences in the presence of putative homologues of clusters and genes implicated in HMO metabolism, consistent with previous reports [6, 53].

Genomically, all analysed *B. breve* sequences, including those isolated from Zimbabwean cohort, contained homologues of the lac (BBR_RS18470-BBR_RS18480),
nah (BBR_RS18490-BBR_RS18520), and inp/lgt (BBR_RS18650-BBR_RS18675) clusters previously annotated in B. breve UCC2003 and involved in the metabolism of lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT) (Fig. 3, Supplementary Table S7) [51]. Interestingly, the presence of the B. breve int HMO cluster (BBR_RS13075-BBR_RS13100) was inconsistent in analysed genomes. Sequences from the USA contained homologues of this cluster, while partial homology was detected in strains from Zimbabwe.

Fig. 3 The presence and absence of homologues of known HMO degradation clusters in Bifidobacterium isolates
The ability of type strain \textit{B. infantis} ATCC 15697\textsuperscript{T} to degrade LNT and LNnT is regulated by a global transcription factor NagR acting as a regulator for multiple gene clusters, including \textit{nag} (Blon\_0879-0885), \textit{lnp} (Blon\_2717-2177) and H1 (BLON_RS12070-BLON_RS12215) \cite{49, 54}. We identified homologues to the \textit{nag} and/or \textit{lnp} clusters in all analysed \textit{B. infantis} and \textit{B. longum} genomes, and partial homology in all \textit{B. breve} sequences, while the majority of \textit{B. infantis} sequences only contained partial H1 cluster, including those from the Zimbabwean cohort (Fig. 3, Supplementary Table S7).

Degradation of fucosylated HMOs by bifidobacteria has been linked to the presence of particular enzymatic machinery containing an alpha1-3/4-fucosidase (GH29) and/or alpha-1–2-fucosidase (GH95). A specific gene cluster for 2’-fucosylactose (2’-FL) metabolism has been described in \textit{B. longum} SC569, (BLNG\_01254-BLNG\_01264), with homologous genes identified in \textit{B. infantis} ATCC 15697\textsuperscript{T} and \textit{B. pseudocatenulatum} DSM 20438\textsuperscript{T} \cite{52}. In our dataset, the majority of \textit{B. infantis} genomes contained genes homologous to those previously implicated in the utilisation of fucosylated HMOs, including the key GH29 and GH95 fucosidases, while \textit{B. breve} genomes were generally missing the GH29 gene, except for the Zimbabwean strain LH\_ZT\_59 (Fig. 3, Supplementary Table S7).

Another type of decorated HMOs, sialylated oligosaccharides, require sialidases for degradation. Extracellular enzymes belonging to this group, such as SiaBb2 identified in \textit{B. bifidum} ATCC 15696, allow sialylated HMOs to be digested on the bacterial cell surface \cite{55, 56}, while intracellular sialidases have been identified in \textit{B. infantis} ATCC 15697\textsuperscript{T} \cite{49}. Our analysis identified homologues of sialidases in all \textit{B. infantis} genomes; with over 95% identity to that from \textit{B. infantis} ATCC 15697\textsuperscript{T} suggesting that these strains may be able to perform intracellular digestion of sialylated HMOs (Supplementary Table S7).

### Distribution of antibiotic resistance genes in infant-associated genomes

The presence of antibiotic resistance genes (ARGs) in bacterial strains has been considered a significant threat to human health in recent years. Many genes can confer resistance, but factors such as the abundance and the transmissibility of ARGs are important in evaluating the relative health risks. To take a global view of the distribution of ARGs in our infant-associated genomes, we examined their abundance and composition using the CARD database. This approach identified a total of 30 putative ARGs potentially capable of conferring resistance to 11 antibiotic classes in 78 out of the 81 genomes included in the analysis. Across the dataset, \textit{Bifidobacterium} genomes harboured genes potentially capable of conferring resistance to aminoglycosides, dianimopyrimidines, macrolides, sulfonamides, rifamycin and tetracyclines, while this list was more extensive in enterococci and additionally included phenicols, phosphonic acid antibiotics, nucleoside antibiotics, glycopeptide antibiotics and antiseptic agents (Fig. 4). On average, \textit{Bifidobacterium} genomes contained 1.4 ARGs (range 1–3), compared to 4.85 on average for \textit{Enterococcus}, with \textit{E. faecalis} sequences harbouring a slightly higher number of genes than \textit{E. faecalis} (5 on average with range between 3–9 and 4.74 on average with range between 3–10, respectively) (Supplementary Table S8).

In terms of the distribution of the ARGs in analysed genomes, we observed strain- rather than species- or location-specific differences in \textit{Bifidobacterium}. The rifamycin-resistant beta-subunit of RNA polymerase (\textit{rpoB}) was detected in 98.8% (\textit{n} = 77) of those bifidobacterial genomes that contained detected ARGs, while the tetracycline resistance genes \textit{tet(W)} and \textit{tet(O)} were the second most abundant gene class identified across bifidobacterial species present in 12.8% of sequences (\textit{n} = 10). Further 5.1% of genomes (\textit{n} = 4) belonging to 3 different \textit{Bifidobacterium} species contained \textit{ermX} gene known to confer resistance to erythromycin (Fig. 4, Supplementary Table S8) \cite{57}. These observations contrasted those made for enterococci, with species-specific differences in ARGs distribution between \textit{E. faecium} and \textit{E. faecalis} clearly identifiable. \textit{E. faecium} genomes, unlike those of \textit{E. faecalis}, all harboured aac(6')-li gene conferring resistance to aminoglycosides and \textit{vanY} gene responsible for conferring resistance to vancomycin, while \textit{E. faecalis} genomes were characterised by the global presence of \textit{drfE} (resistance to trimethoprim), \textit{efrA} (resistance to rifamycin, fluoroquinolone, and macrolides) and \textit{vanT} genes (resistance to vancomycin). Genomes of Zimbabwean \textit{E. faecalis} isolates were among those harbouring an above average number of ARGs (\textit{n} = 9 and \textit{n} = 6), and interestingly, they were the only ones harbouring genes potentially conferring resistance to dianimopyrimidines. In the \textit{E. faecalis} group, the higher-than-average number of ARGs was identified in 3 out of 6 genomes from Zimbabwe (Fig. 4, Supplementary Table S8).

### Discussion

This study provides preliminary insights into the genomic makeup of specific strains of \textit{Bifidobacterium} and \textit{Enterococcus} recovered from an infant cohort from Zimbabwe, and explores some of the properties that allow these organisms to thrive in the infant gut environment, particularly their carbohydrate utilisation potential, including HMOs. It also examines the presence of
putative antibiotic-resistance genes in isolated strains. This knowledge can be leveraged to develop novel probiotics or prebiotics that support the growth of beneficial bifidobacteria and enterococci in infants, potentially leading to improved gut health and immune function [58]. This study may help shape policies on infant health, antimicrobial resistance monitoring, and clinical efforts related to the gut microbiota in Zimbabwe.

The *B. longum subsp. infantis* group, the Zimbabwean isolates clustered either together or with the type strain *B. infantis ATCC 15697T*. This clustering suggests a closer relationship among strains from similar geographical locations or between the Zimbabwean isolates and the type strain from the United States. The distances to strains from Tanzania and Mozambique were shorter compared to those from the UK and USA, indicating a possible regional pattern within this taxonomic group.

Our analysis indicated differences in the gene content related to carbohydrate metabolism among bifidobacterial and enterococcal species, reflected in the varying numbers of genes belonging to different GH families. These findings emphasise the genetic diversity within and

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**Fig. 4** The predicted distribution of antimicrobial resistance genes in isolates
between bacterial species, with potential implications for their metabolic capabilities and ecological roles within the infant gut microbiota [35, 36, 59]. The differences observed in the proportion of genes assigned to categories related to metabolism, information storage, and processing in Bifidobacterium and Enterococcus may also help when developing and designing targeted pre- and probiotics, and targeted treatments.

Additionally, the analysis of putative protease activity revealed consistent representation of gamma-glutamyl hydrolases, suggested to act as endopeptidases on gamma-linked glutamate bonds of gamma-polyglutamate substrates such as folates and anti-folates, in Bifidobacterium [60]. The presence of these enzymes indicates the potential involvement of Bifidobacterium in the metabolism of folates and anti-folates, responsible for growth, development, and immune system function [60]. On the other hand, Enterococcus genomes were characterised by highest abundance of aspartyl peptidases previously implicated in antimicrobial resistance and virulence [14, 61].

HMOs play diverse and essential roles in supporting infant health and development, from promoting a healthy gut microbiota [62] and modulating immune responses [63] to potentially influencing brain development and reducing the risk of allergies [64]. Therefore, identification of strain- and species-specific differences in bifidobacterial potential for HMO metabolism in this study, implies the importance of acknowledging that probiotic strains may differ in their ability to utilize HMOs, which could impact their overall efficacy in promoting gut health and supporting immune development in infants [65]. These findings have implications for the design of future studies investigating the role of HMOs in shaping the gut microbiota and influencing host health outcomes in Zimbabwe.

When influencing health outcomes in developing countries like Zimbabwe, where malnutrition is prevalent [66], the right HMO composition can have a significant impact on children’s health outcomes [67]. However, the composition of HMOs in breast milk varies among mothers, and some structures are more easily metabolized by specific Bifidobacterium strains. Some HMO structures, such as 2’-fucosyllactose (2’-FL) and lacto-N-neotetraose (LNNt), promote the growth of Bifidobacterium longum subsp. infantis which are associated with improved health outcomes [68]. Implying, the right HMO composition can promote the growth of beneficial bifidobacteria. The presence or absence of 2’-FL in breast milk due to maternal secretor status can influence the establishment and abundance of Bifidobacteria in the infant’s gut microbiota. In this study B. longum subsp. infantis was present in some strains found in Zimbabwe stool samples. This could reduce the risk of infectious diseases and improve nutrient absorption, leading to better health outcomes for the infants [68].

In our study, we further compared the distribution of antibiotic resistance genes (ARGs) in Bifidobacterium and Enterococcus in our dataset. Consistent with previous findings [15, 69, 70], our results indicate the presence of resistance genes against various classes of antimicrobial drugs in both bifidobacteria and enterococci, with Enterococcus genomes containing a higher number of ARGs compared to Bifidobacterium, on average. The identification of a diverse range of ARGs capable of conferring resistance to multiple antibiotic classes highlights the need for careful consideration when prescribing antibiotic therapy for pediatric patients [71]. The presence of ARGs in Bifidobacterium species can hinder their approval as probiotic strains, limiting further research into their beneficial potential. Furthermore, E. faecium isolates from Zimbabwe exhibited genes conferring resistance to diaminopyrimidine antibiotics that were not found in the isolates from Europe and North America. This could indicate a potential regional variation in antimicrobial resistance patterns. Understanding local differences in antimicrobial resistance is crucial for informing targeted interventions such as for instance, the One Health approach and increased surveillance efforts [72].

The strength of the study comes from the fact that it represents the first investigation of its kind in the Zimbabwean context. We aimed at identifying putative beneficial genomic properties of locally sourced bacterial strains and their antibiotic resistance profiles. This initial exploration is crucial for the prospect of utilizing possibly probiotic strains to bolster infant gut health in Zimbabwe. The collaborative nature of the study was quite significant, as researchers from various institutions and disciplines worked together, fostering innovative thinking and knowledge exchange.

The study’s sample size for bacterial isolation from the Zimbabwean cohort was a limitation, which affected the generalizability of the findings to the broader population of Zimbabwean infants. However, this small sample size provided a preliminary view of what could be expected in the Zimbabwean samples. Moving forward, continuing isolation work, in an effort to assemble a larger collection of prospective beneficial candidate strains and subject them to further genomic and phenotypic screening, needs to be undertaken. A number of important aspects relating to the safety of bacterial strains have not yet been addressed by this study. These include, among others, the presence of putative insertion sequences and mobile genetic elements, and the potential for transferability of detected ARGs to other microbiota members. In addition, evaluating antimicrobial susceptibility of isolates
would be a crucial step in assessing their suitability as candidate strains. In the future, investigations of a larger geographical area and the focus on Africa, rather than just Zimbabwe, would be needed to provide a clearer picture of the potential of locally sourced microbiota members to confer health benefits.

Conclusion
Strains displaying potentially beneficial properties have been isolated and identified from Zimbabwean infants, which could be considered for the development of early-life diet and microbial therapies. However, the presence of antibiotic-resistance genes in infant-associated microbiota is concerning due to the potential implications for individual and public health. Enterococcus isolates exhibit a wide range of antimicrobial resistance genes. The presence of these genes in both global and Zimbabwean isolates highlights the challenge in treating infections caused by these bacteria.

Abbreviations

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<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tr>
<td>ANI</td>
<td>Average nucleotide identity</td>
</tr>
<tr>
<td>ARG</td>
<td>Antimicrobial resistance gene</td>
</tr>
<tr>
<td>CAZyme</td>
<td>Carbohydrate-active enzyme</td>
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<tr>
<td>CDS</td>
<td>Coding sequence</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>GH</td>
<td>Glycoside hydrolase</td>
</tr>
<tr>
<td>HMO</td>
<td>Human milk oligosaccharide</td>
</tr>
<tr>
<td>LMICs</td>
<td>Low-medium income countries</td>
</tr>
<tr>
<td>LNT</td>
<td>Lacto-N-tetraose</td>
</tr>
<tr>
<td>LNnT</td>
<td>Lacto-N-neotetraose</td>
</tr>
<tr>
<td>MAG</td>
<td>Metagenome-assembled genome</td>
</tr>
<tr>
<td>MRS</td>
<td>De Man, Rogosa, and Sharpe</td>
</tr>
<tr>
<td>RCM</td>
<td>Reinforced Clostridial Medium</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10618-2.

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Authors’ contributions
LJH, DTZ, MK and TEM designed the overall study. TEM and DTZ were responsible for sample collection, whilst IC was the paediatrician responsible. BAT, DTZ, TEM, JM and AF processed the samples and isolated strains. JM and AF prepared the DNA for WGS, MK performed all genomic analysis and visualization and MK and LJH analysed the data. TEM and MK drafted the manuscript, LJH, DTZ, IC, and EG provided further edits and co-writing of the final version. All authors read and approved the final manuscript.

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Availability of data and materials
The draft genomes of 15 infant-associated Zimbabwean isolates sequenced here have been deposited to the NCBI database under the BioProject number PRJNA1036581.

Declarations

Ethics approval and consent to participate
The research adhered to the guidelines outlined in the Declaration of Helsinki, where the participants’ mothers were adequately informed about the study before providing their written consent for their infants to participate. Additionally, the study was initiated following ethical approval from multiple committees, including the Harare City Council Ethics Committee (3/7), the Joint Research Ethics Committee for the University of Zimbabwe Faculty of Medicine and Health Science and Parirennyata Group of Hospitals (353/2021), Medical Research Council in Zimbabwe (MRCZ A/2853), and the Research Council of Zimbabwe reference number 04669.

Consent for publication
The co-authors consented to this manuscript being published.

Competing interest
The authors declare no competing interests.

Author details
1. Faculty of Medicine and Health Sciences, Department of Laboratory Diagnostic and Investigative Sciences, Medical Laboratory Sciences Unit, University of Zimbabwe, Box A 178, Avondale, Harare, Zimbabwe. 2. Faculty of Medicine and Health Science, Department of Biochemistry, Midlands State University, P Bag 9055, Senga Road, Gweru, Zimbabwe. 3. Intestinal Microbiome, ZIEL - Institute for Food & Health, Technical University of Munich, Weihenstephaner Berg 3, 85354 Freising, Germany. 4. Biostatistics and Computational Biology, APC Microbiome Ireland, University College Cork, Cork T12 YT20, Ireland. 5. Microbiome & Health, Quadram Institute Bioscience, Norwich Research Park, Norwich NR4 7UQ, UK. 6. Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK. 7. Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 42TT, UK. 8. Faculty of Medicine and Health Sciences, Department of Family Health, Paediatrics Unit, University of Zimbabwe, Box A 178, Avondale, Harare, Zimbabwe.

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References


60. Rossi M, Amaretti A, Raimondi S. Folate production by probiotic bacteria is governed by global transcriptional regulator NagR. mSystems. 2022;7(5):e003422.


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