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Review

Exploring the Potential Influence of the Human Gut Microbiota on the Gut Resistome: A Systematic Review

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Abstract: Antibiotic resistance is a global health problem. The human gut microbiome is implicated in the dynamics of antibiotic resistance acquisition and transmission, with the gut microbiota thought to play a crucial role. This study aimed to determine the potential influence of the human gut bacteria microbiota on the gut resistome and the relationship between the gut microbiota and *Escherichia coli* resistome. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guideline was used to systematically review studies that characterized the gut microbiota and resistome using metagenomic analysis and/or those that reported gut *E. coli* resistome in healthy individuals. Changes in the diversity and abundance of the bacterial gut microbiota and the resistome across different time points and participant groups were summarized. Additionally, using *E. coli* resistome as a proxy for the gut resistome, the microbiota composition of the gut harboring antibiotic-resistant *E. coli* was examined. The findings suggest that lower bacterial microbiota diversity is likely associated with an increased abundance of the overall gut resistome. Age-related differences were observed, with younger infants exhibiting lower microbiota diversity and higher antibiotic resistance gene (ARG) abundance compared to older infants and adults. Studies that reported positive correlations between the relative abundance of Proteobacteria and ARGs were mainly driven by members within the Enterobacteriaceae family, mainly *E. coli*. This study also reveals that human gut microbiome studies investigating the gut resistome using metagenomic sequencing approaches in healthy individuals are uncommon.

Keywords: gut microbiota; gut resistome; antibiotic resistance genes; *Escherichia coli*

Citation: Fri, J.; Raphalalani, M.; Mavhandu-Ramarumo, L.G.; Bessong, P.O. Exploring the Potential Influence of the Human Gut Microbiota on the Gut Resistome: A Systematic Review. *Microbiol. Res.* **2024**, *15*, 1616–1633. https://doi.org/10.3390/microbiolres15030107

Academic Editor: Maria Pia Franciosini

Received: 17 July 2024
Revised: 12 August 2024
Accepted: 16 August 2024
Published: 21 August 2024

1. Introduction

Antibiotic resistance is a global health challenge that has resulted in continuous reduced antibiotic effectiveness and increasing morbidity and mortality due to bacterial infections [1]. The Sustainable Development Goal (SDG) indicator on antimicrobial resistance aims to lower antimicrobial-resistant infections, including those caused by *Escherichia coli* resistant to third-generation cephalosporins [2]. Knowledge of the factors that increase the emergence and spread of antibiotic resistance is essential in developing strategies and informing policies and procedures for antibiotic resistance stewardship, working toward the SDG goal of increasing well-being and reducing infectious diseases by 2030 [3]. The gut microbiome is an integral part of humans and significantly influences health [4–6]. It comprises a varied ecosystem of microorganisms that co-exist, including bacteria, fungi, and viruses, most of which are beneficial [7,8]. However, the bacteria component of the gut microbiota is the most abundant and functionally diverse, comprising members of the

Phylum Bacteroides, Firmicutes, Actinobacteria, and Proteobacteria, with Bacteriodes and Firmicutes shown to be the most abundant [9–11]. These bacteria play significant roles in digestion, immune system modulation, and metabolic activities. Nonetheless, factors such as diet, infection, chronic diseases, antibiotics and other environmental exposures, may alter the gut bacterial composition, leading to dysbiosis [12–18]. The gut can also operate as a repository of antibiotic-resistant bacteria and genes [7,19,20] that can be disseminated to susceptible bacteria and the environment [21]. Antibiotic resistance may develop through mutations and spread vertically through replication of resistant bacteria or horizontally through conjugation, transformation, and transduction. Bacteriophages and mobile genetic elements, such as plasmids, transposons, and integrons, play major roles in the transfer and, hence, the spread of the antibiotic-resistant genes [22,23]. Antibiotic-resistant bacteria and genes have been reported even in the guts of healthy individuals, including infants who have never been exposed to antibiotics [24–28].

Understanding the gut bacteria resistome is crucial in the context of antibiotic resistance and its implications for human health. The bacteria resistome comprises genes conferring antibiotic resistance that are carried by commensals, including opportunistic pathogens. Therefore, the gut microbiota likely influences the ARG composition and abundance. However, how it influences the resistome remains unclear. It is also unknown if varying microbiota contribute uniformly to antibiotic resistance enrichment. Unravelling these associations between the gut bacteria microbiota and the resistome would be useful in identifying potential targets for controlling antibiotic resistance in humans. It may also serve as a foundation for formulating guidelines and strategies to address antibiotic resistance, including interventions that modify the gut microbiota. Based on these, we hypothesized that the gut bacteria microbiota influences the abundance of gut ARGs.

Next-generation sequencing (NGS), particularly metagenomic sequencing, has improved microbiome characterization and analysis of the total gut resistome pool. Among the normal gut flora, *Escherichia coli* can be used as an indicator organism in healthy populations to understand the risk factors and trends in gut antibiotic resistance. This is because (i) *E. coli* is less fastidious and, therefore, can easily be cultivated in the laboratory; (ii) it is opportunistic, causing not only gastrointestinal infections but also extraintestinal infections at several sites of the human body [29–33], most of which are due to translocation from the gut; (iii) these infections require antibiotic treatment, and broad antibiotic options have been used [34]; and (iv) *E. coli* antibiotic resistance has been reported in many *E. coli*-associated infections, with geographical variations in resistance patterns. Furthermore, the bacterium is versatile, zoonotic, and has been isolated from animals, including livestock, pets, and other primates, which are sources of acquisition for humans [35–37]. Given the significance of *E. coli*, it can be used as a proxy to elucidate the association between the gut microbiome and the resistome. Therefore, we also hypothesized that there is a relationship between the composition of the human gut microbiota and *E. coli* resistome. This review aimed to determine the (i) potential influence of the gut bacterial microbiota on the bacterial resistome and (ii) the relationship between the microbiota composition and *E. coli* resistome. To achieve these, we reviewed published studies investigating the gut microbiota and resistome and those investigating the gut microbiota and *E. coli* resistome in apparently healthy individuals.

2. Methodology

2.1. Search Strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) approach was used to conduct this review, except for meta-analysis (Supplementary Materials, Table S1). To identify potentially relevant articles investigating the human gut microbiota and gut resistome, or the human gut microbiota and *E. coli* resistome, a literature search was performed in Web of Science, Scopus, and PubMed databases using keywords related to gut microbiota, resistome, and *E. coli*. Separate searches were performed to address the two objectives. The detailed search terms, combinations, and

strategies are provided in the Supplementary Materials (Table S2). All fields were searched, and no date restriction was employed. The last search was performed on 25 March 2024. The bibliographic search outcomes were imported to Mendeley desktop v. 1.19.5 and duplicates were removed.

2.2. Selection of Studies

Eligible studies for inclusion were those that (i) were human studies involving healthy participants that investigated the gut bacteria microbiota and resistome or the gut microbiota and *E. coli* resistome; (ii) identified gut microbiota and resistome by metagenomic sequencing; and (iii) were primary research articles published in English. Studies of the following nature were excluded: (i) those that did not characterize the microbiota by metagenomic sequencing; (ii) those that did not report the gut resistome or *E. coli* resistome/resistance; (iii) those that targeted only specific bacteria as microbiota; (iv) those that involved unhealthy or critically ill participants; (v) those that involved high-risk populations likely to carry ARGs, such as studies involving mass administration of antibiotics; (vi) those that involved participants with undefined health status; and (vii) those that involved preterm infants. Only data from full-term infants, if described as part of the latter, were extracted and included in the analysis. Also, studies in which late preterm participants (33–36 weeks) constituted less than 10% of the total sample were included. Reviews, book chapters, case reports, studies in languages other than English, and those that utilized publicly available data (metadata) were also excluded.

2.3. Data Extraction and Synthesis

Variables were extracted from all eligible studies following the inclusion and exclusion criteria into a predefined data extraction sheet. This included the author(s), year of publication, title of study, objective, design, country of study, sample size, age of participants, methodology, sequencing platforms, bioinformatic pipelines, alpha diversity measures, microbiota composition, and *E. coli* resistome. The data for full-term infants were extracted and reported in cases where the studies included preterm and full-term infants. If only a subset of a study was analyzed for the microbiota and resistome, only the sample size of the subset was captured. Supplementary sheets of the included studies were consulted for data missing from the original articles. Information not specified in a study, or the Supplementary Materials was captured as data not reported (NR). Figure 1 represents the PRISMA approach used in sourcing, identifying, and selecting studies included in the analysis.

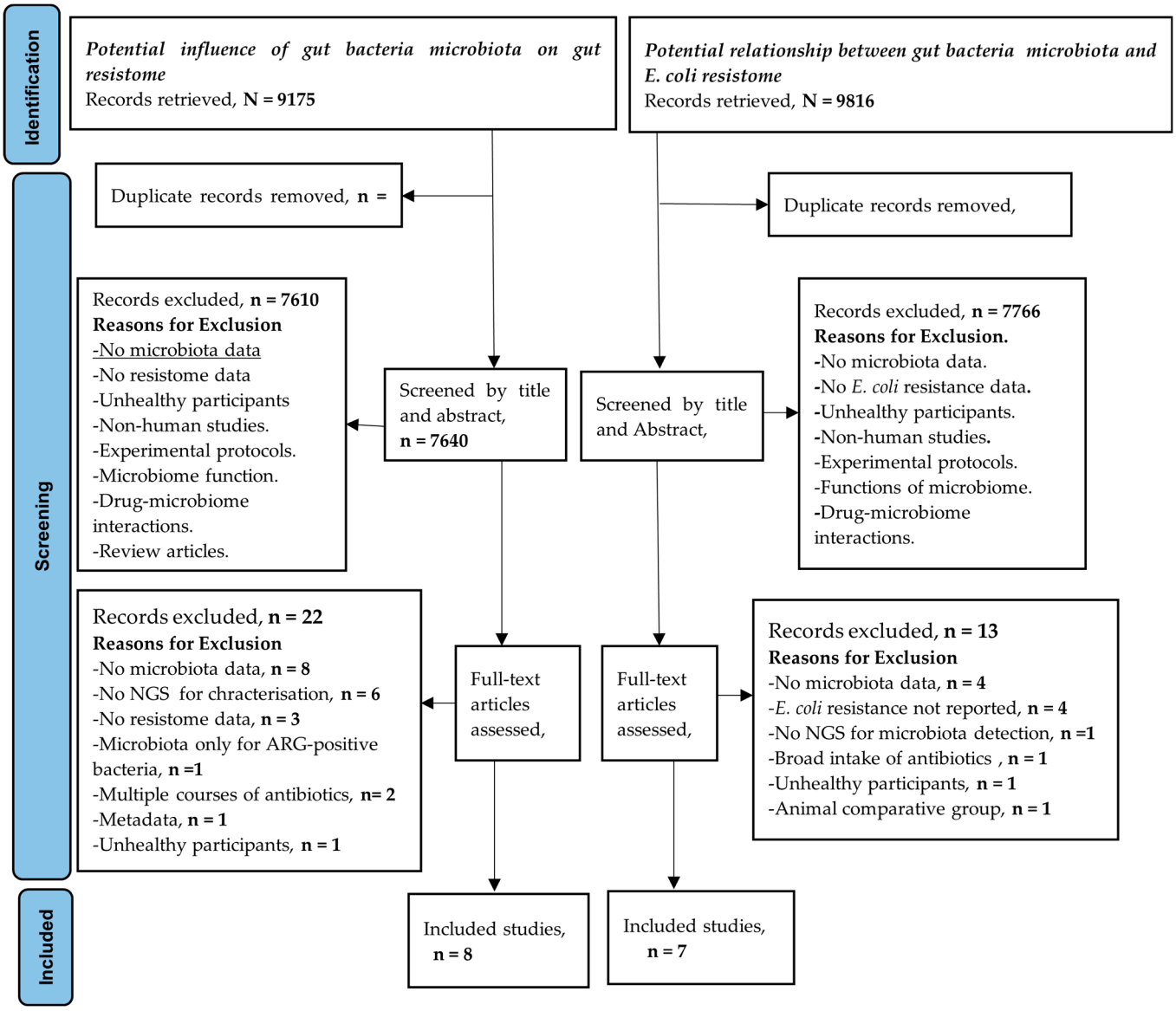

Figure 1. PRISMA flow diagram indicating the screening and final selection of studies used to examine the potential influence of the gut microbiota on the gut and *E. coli* resistome.

2.4. Assessment Parameters

To determine the potential influence of the gut microbiota on the gut resistome, the following outcome measures were considered: significant changes in the microbiota alpha diversity and abundance over different time points; significant changes in the microbiota alpha diversity and the abundance among comparative groups. As a secondary measure, correlations between the microbiota and resistome load, if available, were included.

To determine the potential association between the gut microbiota and *E. coli* resistome, the composition of the bacteria microbiota and the *E. coli* resistome composition were recorded, and homogenous data were summarized. The secondary measure was to examine if they were any reported correlations between the microbiota and the *E. coli* ARGs.

2.5. Risk of Assessment Bias and Critical Appraisal of Eligible Studies

To limit possible bias and errors, the first author (JF) performed the data search, and the second author (MR) performed an independent search, examination, and confirmation. These two independent authors (JF and MR) also performed preliminary screening based on the titles and abstracts and data extraction from full-text articles. Any discrep-

ancies were discussed, and all authors came to a consensus. The Joanna Briggs Institute (JBI) critical appraisal checklist (https://jbi.global/critical-appraisal-tools) (accessed on 29 April 2024) was used to appraise the longitudinal and cross-sectional studies before their final inclusion in the analysis (Tables S3 and S4).

3. Results

3.1. Characteristics of Studies Included in the Analysis

The literature search to identify articles to assess the potential influence of the human gut microbiota on the gut resistome yielded a total of 7640 studies that were subjected to screening. Following screening, only a small proportion of the studies (0.1%, 8/7640) fulfilled the study eligibility [38–45]. Five of the eight (62.5%) eligible studies were longitudinal, out of which four involved child participants within the first five years of life. Three of the eight (37.5%) studies were cross-sectional. Three studies were conducted in the USA, two in China, and one in Norway, Denmark, and Vietnam. The majority (75%, 6/8) of the studies used the MetaPhlAn bioinformatic database to profile the microbiota. Various databases were used for post-sequence analysis to characterize the gut resistome, and the protein markers were primarily sourced from the Comprehensive Antibiotic Resistance Database (CARD). The approaches used for the gut microbiota and resistome characterization in the eligible studies are shown in Table 1 and comprehensive details of the databases, pipelines and tools for bioinformatic sequence analysis are presented in Tables S5 and S6.

Table 1. Summary of characteristics of included studies, testing methodology, and sequencing strategies.

S/N	Country of Study	Study Design	Study Objective/Hypothesis	Age of Participant and Study Time Points	Sample Size	DNA Extraction and Quantification	Sequencing Technology and Platform	Major Bioinformatic Database and Pipelines	Ref.
1 [ab]	Norway	Longitudinal	Determine resistome and mobilome across gestational ages and microbiota-modifying treatment.	7 days, 28 days, 120 days, 365 days.	n = 10	NorDiag Arrow Stool DNA Extraction kit (+bead beating); Qubit, Nanodrop.	Shotgun Illumina Miseq	Bowtie2; MetaPhlAn3 (based on CHOCOPhlAn); MetaSPAdes and MetaQUAST (from QUAST); ShortBRED based on CARD; NanoARG; HUMAnN.	[38]
2 [ab]	USA	Longitudinal	Determine factors associated with early life resistome development.	6 weeks and 1 year.	n = 195	Fecal DNA extraction kit; Qubit.	Shotgun	MetaPhlAn; PanPhlAn; HUMAnN2; ShortBRED based on CARD.	[39]
3 [ab]	USA	Longitudinal	Determine potential sources of infant and maternal ARGs.	Mother and child; 1 month, 6 months.	n = 10	InviMag® Stool DNA Kit; Qubit, Nanodrop.	Shotgun Illumina NextSeq	Bowtie2; MetaPhlAn2; CARD; ResFinder; PlasmidFinder.	[40]
4 [ab]	Denmark	Longitudinal	Characterize the ARGs acquired during the first year of life and assess the impacts of diverse environmental exposures on ARG load.	1 year 1 week 1 month 1 year 4 years 5 years	n = 662—shotgun n = 660—16S rRNA	PowerMag Soil DNA Isolation Kit.	Shotgun Illumina NovaSeq 16SrRNA sequencing-Illumina Miseq	SPAdes; HUMAnN2; MetaPhlAn; MetaWRAP; MetaBAT2; Bowtie2; QIIME2. CARD.	[41]

Table 1. *Cont.*

S/N	Country of Study	Study Design	Study Objective/Hypothesis	Age of Participant and Study Time Points	Sample Size	DNA Extraction and Quantification	Sequencing Technology and Platform	Major Bioinformatic Database and Pipelines	Ref.
5 [a]	China	Longitudinal	To understand the characteristics of the gut microbial composition.	18–69 years (mean = 28.6)	n = 7 (followed monthly for 1 year)	QIAamp Fast DNA Stool Mini Kit.	Shotgun Illumina HiSeq	HUMAnN3; UniRef90; KEGG; Kraken2; ResFinder; SPAdes.	[42]
6 [a]	Vietnam	Cross-sectional	Healthy human gut in Vietnam is a source of ARGs transferable to gut pathogens.	0–23 months 2–5 years >18 years	n = 42	FastDNA soil kit.	Shotgun Illumina	Bowtie2; Kraken2; Bracken; ARGANNOT database.	[43]
7 [a]	USA	Cross-sectional	To characterize the microbiome and resistome of dairy workers.	Mean age of dairy workers = 38.4; Mean age of community controls = 49.5	n = 16 (10 dairy workers and 6 non-dairy workers	MoBio DNeasy PowerLyzer PowerSoil Kit.	Shotgun Illumina HiSeq	MetaPhlAn3; ChocoPhlAn; Anvio; Centrifuge; MEGAHIT; ABRicate; MetaCherchant; Kraken2; CARD.	[44]
8 [a]	China	Cross-sectional.	Determine antibiotic resistome shared between chicken farms and live poultry market workers and those with no contact with live poultry markets.	NR	n = 36 (18 live poultry market workers and 18 non-workers)	DNeasy PowerSoil Pro Kit; Agarose gel electrophoresis; Qubit dsDNA assay kit;	Shotgun Illumina NovaSeq PE150.	MEGAHIT; MetaGeneMark: MetaPhlAn2; CARD. ResFinder	[45]
9 [b]	Malaysia	Cross-sectional	Profile the gut resistome of Malaysians and investigate its association with demographic and lifestyle variables.	≤90 years Lower boundary, NR	n = 200	QIAamp PowerFecal Pro DNA Kit.	Shotgun Illumina NovaSeq	BioBakery3; KneadData; MetaPhlAn3; Bowtie; ARGs-OAP.	[46]
10 [b]	USA	Cross-sectional	Characterize fecal, oral, and skin bacterial microbiome and resistome of the Yanomami Amerindians with no previous contact with Western people.	4–50 years old	n = 12	PowerSoil DNA Isolation Kit.	V4 region of the 16SrRNA Illumina HiSeq	PICRUSt STAMP; KEGG; CONCOCT; PARFuMS; Resfams.	[47]
11 [b]	Saudi Arabia	Cross-sectional	To assess pregnancy-induced gut microbiome composition and antimicrobial resistome in Saudi females.	Mean age: NP 39.1 ± 7.7; First trimester: 25.4 ± 4.1; Third trimester: 33.3 ± 7.3.	n = 24 (8 NP, 8 first trimester, 8 third trimester)	QIAamp Fast DNA Stool Mini Kit.	16S rRNA Illumina MiSeq	PANDAseq	[48]

Table 1 summarizes the characteristics of the included studies to investigate the potential influence of the gut bacterial microbiota on the overall gut resistome, and the link between the composition of the gut microbiota and the resistome of *E. coli*, a crucial indicator organism for understanding gut antibiotic resistance trends. The superscript 'a' denotes the studies eligible for objective 1; 'b' denotes the studies eligible for objective 2 and studies marked with 'ab' were eligible for both objectives 1 and 2. NR; Not reported.

The literature search performed to source articles to determine the relationship between the bacteria gut microbiota and *E. coli* resistome yielded a total of 7786 studies subjected to screening. Screening also revealed a small proportion (0.09%, 7/7786) of eligible articles to address the second objective [38–41,46–48]. These constituted four longitudinal and three cross-sectional studies (Table 1). The gut *E. coli* resistome was identified from these studies by shotgun metagenomic sequence analysis (n = 5), functional selection using specific antibiotics (n = 1), and the use of both the Kirby–Bauer disc diffusion technique and PCR detection targeting 47 ARGs in gut isolates.

Generally, four out of the eight studies eligible for objective 1 that explore the influence of gut bacterial microbiota on the gut resistome were also among the seven studies eligible for objective 2, which was set to determine the relationship between the microbiota and the *E. coli* resistome (Figure 1). The publication dates of the eligible studies ranged from 2015 to 2023. The Joanna Briggs Institute (JBI) critical evaluation outcomes were high (72.7–100%) across the included studies, indicating strong study designs and reliable outcomes (Tables S3 and S4).

3.2. The Potential Influence of the Gut Microbiome on the Gut Resistome

To determine the potential influence of the gut microbiota on the gut bacterial resistome, changes in the alpha diversity in the gut microbiota between participant groups or longitudinal sample time points were summarized comparable to the changes observed in the resistome between these groups. Correlations between the microbiota and resistome from individual studies were also extracted with the aim of performing a meta-analysis. However, there was high dissimilarity in the populations based on factors such as age, environmental exposures [39,41,45], and other significant heterogeneity, including the use of different correlation metrics [41,45]. Some studies reported correlations between differential taxa and ARG load [38,39], while others focused on the total gut microbiota and ARGs [41,45]. Additionally, in some cases, strong correlations were reported without providing detailed coefficients [38,40], which are necessary for quantitative analysis. Due to these, a robust meta-analysis was not conducted. Therefore, the results from individual studies were summarized, and the observed trends and consistent findings across the studies are reported as a narrative synthesis.

Except for one of the studies wherein data from an adult group were compared to data from the Human Microbiome Project, all other studies that showed a lower diversity of bacteria microbiota compared to a comparison group also reported relatively higher resistome abundance [38–41,43,45]. Therefore, lower gut microbiota diversity is likely associated with a higher gut ARG gene load. This association was clearly visible when considering age or longitudinal study time points [38,39,43]. Neonates and younger infants (less than 24 months) had a less diverse microbiota, shown to mature over time [43], particularly within the first two years, compared to older infants (Table 2). Concurrently, the gut ARGs were relatively more abundant in the younger than the older participants. This association was reported in five groups from four studies [38–40,43] (Table 2).

Table 2. Bacteria microbiota and potential influence on gut ARGs.

Groups	Metric	Microbiota Diversity	Taxonomic Abundance	ARG Abundance	Associations between Microbiota and Resistome	Ref.
Full-term infants at 7 days, 28 days, 120 days, and 365 days.	α, Shannon diversity	Lowest at 7 days and increased to 365 days.	*Bifidobacterium:* highest at 28 days > 120 > 7 > 365. *Escherichia*: 7 days > 120 > 28. *Bacteroides*: 365 days > lower time points. *Klebsiella*: highest at 7 days similar to 120 days > 28 days, lowest at 365 days.	Higher ARGs at 28 days compared to 120 days. Median: 7 days > 28 days > 120 days.	*E. coli* associated with highest ARGs, followed by *Klebsiella pneumoniae* and *Klebsiella aerogenes.*	[38]
6 weeks vs. 1 year.	α, Shannon and Simpson			Higher	Proteobacteria: positive correlation of resistome composition. *E. coli*: strong positive correlation between *E. coli* abundance and resistome load.	[39]
0–23 months vs. 2–5 years.	α, Shannon	Lower	Proteobacteria and Actinobacteria: higher in children than adults. Bacteroides and Firmicutes A: higher in adults than children	Higher		[43]
0–23 months vs. >18 years.		Lower		Higher		
1-month infants vs. mothers and 6-month infants vs. mothers.	α, Simpson	Lower diversity in infants than mothers.	Higher Gamma Proteobacteria. Higher *E. coli.*	Higher in 1 month. Higher in 6 months.	Gammaproteobacteria: strong positive correlation with resistome load. *E. coli*: strong positive correlation with resistome abundance; strongest predictor of ARGs in infants. *Bifidobacterium*: negative correlation with resistome load.	[40]
NA	OTU richness For *E. coli*		Higher abundance of *E. coli* from one week, lowering to 1 year.	Higher in Proteobacteria. Highest in *E. coli.* Higher in the first year, and lower toward an equilibrium.	Lower gut microbiome maturity associated with higher ARGs. Higher *E. coli* abundance associated with lower gut maturity.	[41]
18–69 years vs. HMP data set.	α, Shannon	Higher		Higher		[42]
Poultry vs. non-poultry workers.	α, Simpson	Lower		Higher		[45]
Dairy vs. non-dairy workers.	α, Shannon		No significant difference.	Lower		[44]

HMP; Human Microbiome Project.

Based on the composition of various microbiota taxa, there was evidence of positive strong correlations between the microbiota compositional structure and the ARG gene load [38–41]. A strong positive correlation between the compositional relative abundance of Proteobacteria and gut ARG abundance was reported in two studies [39,40]. Also, amongst the Proteobacteria, *E. coli* was reported to have the strongest positive association with resistome abundance [28–41,46,48], followed by other members of Enterobacteriaceae, including *Klebsiella pneumoniae*, *K. aerogenes*, *Citrobacter*, and *Enterobacter* [38]. *E. coli* was identified as the strongest predictor of ARGs in infants, and according to Li et al. [41], it harbored over 51% (68/133) of the different ARGs in the gut of young infants. Conversely, there was a negative correlation between Bifidobacterium and ARG load.

Although the potential influence of external factors was not considered as part of the core objective of this study, two of the eight eligible studies involved healthy participants exposed to different environments known to potentially enhance antibiotic resistance acquisition: the study of Wang et al. [45] involved participants who were exposed to live poultry markets, and that of Trinh et al. [44] involved participants exposed to dairy. Although both those exposed and unexposed to live poultry markets were healthy residents within the same geographical area as the control group, the unexposed group exhibited a higher phylogenetic diversity but lower corresponding ARG diversity and abundance than the exposed, similar to the previous trend described (Table 2).

3.3. The Potential Association between the Gut Microbiota and E. coli Resistome

To examine if there was an association between the gut microbiota composition and *E. coli* resistome, using *E. coli* resistome as a proxy for the gut resistome, seven studies reported resistance in *E. coli* and characterized the gut microbiota by metagenomic sequencing. Generally, there was (i) a significantly low level of detail or comprehensive description provided for gut *E. coli* ARGs and the gut microbiota composition and (ii) high heterogeneity in the microbiota and the corresponding *E. coli* resistome data (Table S7). The data also covered a wide range of participant groups, including infants (7 days old, 6 weeks to 1 year), young children (1 to 5 years), groups dominated by adults and elderly (<90), as well as pregnant and non-pregnant women.

Despite the significant variations and heterogeneity in the data from the seven studies, which reduced the feasibility of drawing concrete conclusions about the relationship between the gut microbiota composition and the *E. coli* resistome, we managed to summarize the composition of *E. coli* resistome and the associated bacterial microbiota based on a subset of more homogeneous data. *Bifidobacterium*, followed by *Escherichia*, was identified as the most abundant genera in the guts of participants less than six months old. The genera *Bifidobacterium* and *Prevotella*, *Collinsella*, *Eubacterium*, and *Ruminococcus*, belonging to Actinobacteria, Bacteroides, and Firmicutes, were the most abundant in the adult-dominated group. It was observed that while *Escherichia* and other members of the Enterobacteriaceae formed part of the top five most abundant genera in the infant groups, *Escherichia*, particularly *Escherichia coli*, was not part of the top five most abundant genera in the adult-dominated group. Khan et al. [48] also presented bacteria families in non-pregnant adult women, where those belonging to the Proteobacteria phylum were not part of the most abundant five. Table 3 summarizes the most abundant genera in the gut of infants and adults that displayed *E. coli* antibiotic resistance.

Table 3. Genus-level microbiota composition of the most abundant genera identified in the guts of apparently healthy participants carrying antibiotic-resistant *E. coli*. This is based on data from Bargheet et al. [38], Dwiyanto et al. [46], and Pärnänen et al. [40].

Infant Groups			Adult-Dominated Group
7 Days Old	**1 Month**	**6 Months**	**<11 Years (2%) 11–20 Years (22%) 20–90 Years (76%)**
Bifidobacterium [A]	*Bifidobacterium* [A]	*Bifidobacterium* [A]	*Bifidobacterium* [A]
Escherichia [P]	*Escherichia* [P]	*Escherichia* [P]	*Prevotella* [A]
Klebsiella [P]	*Lactobacillus* [F]	*Blautia* [Ba]	*Collinsella* [B]
Vellionella [Ba]	*Bacteroides* [B]	*Bacteroides* [B]	*Eubacterium* [F]
Bacteroides [B]	*Streptococcus* [F]	*Lactobacillus* [F]	*Ruminococcus* [F]
Enterococcus [F]	*Staphylococcus* [F]	*Eubacterium* [P]	*Escherichia* [P]
Staphylococcus [F]	*Blautia* [Ba]	*Akkermansia* [V]	*Lactobacillu* [F]

The superscripts indicate the bacteria Phyla: [A]—Actinobacteria; [P]—Proteobacteria; [F]—Firmicutes; [B]—Bacteroides; [Ba]—Bacillota; [V]—Verrucomicrobia.

The summary findings from the studies involving 0–1-year-old participants revealed the multidrug resistance efflux pumps as the most diverse and abundant group of *E. coli* ARGs, followed by ARGs encoding resistance to Beta-lactams, polypeptides, and fosfomycins. Table 4 summarizes the composition of *E. coli* resistome identified from the studies. The resistome profiles and detailed associated gene products are documented in the Supplementary Materials, Table S8.

Table 4. Summary of the composition of gut *E. coli* resistome in 7-day-old to 1-year-old infants. This is based on data from Bargheet et al. [38] and Lebeaux et al. [39].

Antibiotic Resistance Group	Antibiotic Resistance Genes
Multidrug-resistant (MDR) efflux pumps and regulators	*acrA*, *acrD*, *acrE*, *acrF*, *acrR*, *mdfA*, *mdtE*, *mdtF*, *mdtG*, *mdtH*, *mdtO*, *emrA*, *emrE*, *marA*, *marR*, *gadW*, *gadX*, *soxS*, *soxR*, *tolC*,
Betalactam	*ompA*, *ompF*, *ampH*, *EC15*
Polypeptide	*bacA*, *eptA*, *pmrF*
Fosfomycins	*murA*, *glpT*, *uhpT*
Multidrug resistance	*evgA*,
Rifampicin	*rpoB*
Nitrofurans	*nfSA*
Aminocoumarins	*gyrB*
Folate pathway inhibition	*folP*
Peptides	*yojl*

4. Discussion

The current emergence, acquisition, and spread of antibiotic resistance are alarming and need urgent attention. The SDG indicator on AMR aims to lower antimicrobial-resistant infections, working toward achieving the SDG goal of increasing well-being and reducing infectious diseases by 2030 [3]. The gut microbiome, particularly that of a developing infant, is crucial to human health. Belonging to this dynamic consortium, gut resistome studies are vital to continuously unravel important data that aid in developing strategies to reduce the spread of antibiotic resistance. This review provides insights into the gut microbiota that potentially influence the abundance of the gut resistome.

A healthy human gut is thought to have a properly balanced and diverse bacteria composition, and the core microbiota has co-evolved with humans [8,11,49]. It plays a vital role in various activities beneficial to health [50]. This review highlights that (i) a relatively lower diversity of the gut bacteria microbiota is concurrent with a higher relative abundance of the gut resistome [38–41,43,45]; (ii) an increase in the abundance of the gut resistome is mainly due to the increase in the relative abundance of Proteobacteria, particularly Enterobacteriaceae [39–41]; and (iii) among the Enterobacteriaceae, *E. coli* is the primary source of ARGs [38–40]. Also, due to the low gut microbiota maturity and resulting lower diversity in infants, a higher relative abundance of Proteobacteria compared to older age groups is prevalent and associated with abundant ARGs that reduced over time. Generally, following delivery, the neonatal gut is abundant in oxygen; therefore, the presence of strict anaerobes is unlikely. Compared to most gut enterotypes, which are obligate anaerobes, Proteobacteria are facultative anaerobes and, therefore, tolerate the oxygen-rich neonatal gut, justifying the disparity in gut Proteobacteria abundance between newborn infants and older children and adults. Proteobacteria are thought to be essential for the consumption of oxygen and the reduction of redox potential. Thus, they prepare the gut for colonization by the more stable strict anaerobes [51]. It is worth noting that Proteobacteria, particularly those of the gamma-Proteobacteria group, are also significantly involved in conjugation, and hence, horizontal gene transfer [21]. This characteristic increases the odds of disseminating antibiotic-resistant genes to other gut bacteria species, increasing the gut resistome. Therefore, the prevalence of gut ARGs in neonates could be reduced by regulating the relative abundance of Proteobacteria, particularly *E. coli*, thus reducing further dissemination of ARGs to other gut bacteria and the environment.

The findings of this study, which highlights Enterobacteriaceae as a significant contributor to ARG load, mirrors the WHO's report on global deaths attributed to and associated with bacterial antimicrobial resistance, which ranks antibiotic-resistant *E. coli* as the leading pathogen associated with global deaths [52]. Also, out of the approximately 700,000 deaths worldwide that are attributed to antibiotic-resistant infections, approximately 200,000 of these occur in neonates in the first four weeks of life [50]. Increased global deaths are due to *E. coli* that are resistant to third-generation cephalosporins, fluoroquinolone-resistant *E. coli*, and other Gamma Proteobacteria, such as carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Klebsiella pneumoniae*, and third-generation cephalosporin-resistant *Klebsiella pneumoniae* [52].

Although this review highlights Proteobacteria, and particularly *E. coli*, as the major gut bacteria strains harboring ARGs, the possibility of bias cannot totally be refuted, which may also account for a proportion of the identified ARGs linked to *E. coli*. Like other databases, ARG databases, such as CARD and Restfinder, may overrepresent markers frequently identified in certain bacterial species, such as those encoding resistance in *E. coli*. Historically, *E. coli* has been extensively studied and is still a focus of research, and its associated ARGs are undoubtedly inclusive with available genomic data. As a result, the database may contain more known markers for ARGs associated with *E. coli* than for other gut bacteria.

Various environmental exposures may influence the gut microbiota, consequently influencing the resistome. Two studies examined the microbiome and overall gut resistome in participants exposed to dairy and poultry. One [44] found no significant difference in the taxonomic abundance between dairy and non-dairy workers and a lower ARG load in dairy compared to non-dairy workers, likely attributed to the lower sequencing depth. On the other hand, Wang et al. [45] reported significantly less diverse but higher ARGs of live poultry market workers compared to the control group, probably due to environmental exposures. The study also indicated that beta-lactam and lincosamide resistance genes were more prevalent in live poultry market (LPM) workers compared to the control group, and the mechanisms responsible for antibiotic inactivation were found at higher levels in the samples of the LPM workers. It is worth noting that poultry is one of the agricultural sectors with high usage of antibiotics as growth promoters, and a correlation has been shown

between the abundance of human gut ARGs and antibiotic exposure in animals [53]. A study conducted in China, covering 88 poultry farms [54], identified Amoxicillin as the most used antibiotic (76.5%), followed by norfloxacin, ofloxacin, ceftriaxone, and oxytetracycline. The increased use of beta-lactam antibiotics in poultry is likely a contributing factor to the higher prevalence of corresponding ARGs in LPM workers, who are regularly exposed to these environments, compared to the unexposed group. Exposure to antibiotics increases microbiome dysbiosis, ultimately decreasing diversity and leading to a less stable gut microbiota composition and increased ARGs, primarily due to Proteobacteria enrichment. A previous study reported an increase in the diversity and abundance of ARGs in participants exposed to antibiotics within six months prior to the commencement of the study compared to a lower diversity in the antibiotic-unexposed individuals [55]. Specifically, the relative abundance of *E. coli*, at 0.1% in the antibiotic-unexposed group, increased to nearly 10% in the antibiotic-exposed group.

The healthy human gut microbiota is mainly dominated by the phyla Firmicutes and Bacteroidetes, with lower abundance of Actinobacteria, Proteobacteria, and other minor phyla [9,55]. However, of the Actinobacteria phylum, members of the genus *Bifidobacterium* are the most abundant, dominating the guts of healthy infants, similar to what we observed in participants' guts harboring antibiotic-resistant *E. coli.* However, this is almost always followed by abundance of *Escherichia* or other Proteobacteria genera in infants, as opposed to Actinobacteria or Bacteroidetes genera in adults. Although indicating resistance, a healthy gut dominated by *Bifidobacterium* spp. highlights its importance in modulating and enhancing metabolic and mitochondrial activities [56]. *Bifidobacterium* function in vitamin and protein synthesis, digestion supplementation, immune system stimulation, and suppression of the growth of exogenous organisms [57]. In a previous study by Gagnon and colleagues [58], two strains of *Bifidobacterium* isolated from infant feces were shown to be resistant to bile, acid, and lysozyme and inhibited enterohemorrhagic *Escherichia coli* serotype O157:H7 in vitro, decreasing its adherence to human enterocyte-like CaCo-2 cells. Inhibition of the potentially pathogenic *E. coli* serotype was associated with an increased concentration of the *Bifidobacterium* species [58]. This shows that an increased relative abundance of *Bifidobacterium* would be beneficial to human health, as it suppresses the abundance of the *E. coli* population, resulting in a reduction in *E. coli* antibiotic resistance and overall gut resistance.

The current study also identified efflux pump-associated ARGs as significant contributors to *E. coli* resistome. The mechanisms by which efflux pumps confer resistance vary from intrinsic to overexpression of the pumps or mutations in the repressor genes or other encoding genes [59,60]. These mechanisms successfully lower intracellular concentrations of several antibiotics, a major source of the multidrug-resistance traits observed. For example, the AcrAB efflux pumps confer resistance to multiple antibiotics, including tetracycline, quinolones, and fluoroquinolones [61]. *E. coli*'s central involvement as a multidrug-resistant pathogen increases the gut antibiotic resistance gene pool and subsequent spread. This could be related to its ranking as the leading pathogen associated with global mortality due to antibiotic resistance [52].

Generally, antimicrobial resistance ranks among the most significant risks to global public health linked to approximately five million deaths in 2019, with Western sub-Saharan Africa having the highest number of deaths [52]. The WHO report is particular about generating antimicrobial resistance programs and initiatives, especially in low- and medium-income countries (LMICs). However, it alludes to the fact that these countries are the most severely challenged by the lack of well-characterized data and having huge knowledge gaps that hamper the effective control of antibiotic resistance [62]. In this review, over 7000 articles were screened to answer the core objectives, and approximately only 0.1% of studies were eligible to address each, with minimal representation from LMICs, highlighting a huge a huge dearth of data which align with the WHO report. Also, since the development of NGS in the early to mid-2000s [63], it has been applied in many biological scientific fields, yet the earliest article in the current review was published in 2015, approx-

imately two decades later. It is worth mentioning that unlike culture-based techniques, metagenomic sequencing has the added advantage of profiling the total gut microbiome and resistome and could also reveal circulating novel antibiotic resistance genes (ARGs) and plasmids carrying these genes. The low global uptake indicates that research using innovative technology to generate antibiotic-resistant well-characterized data sets is still uncommon. Therefore, this research area needs to be fostered to provide valuable data for strategic intervention. Although not undermining the significance of nosocomial-acquired antibiotic-resistant infections, clinical cases most often stem from community carriers. If human gut resistome community data are available, including data on how the microbiota evolves with the overall antibiotic resistance or its evolution with the resistome of a targeted species, such as *E. coli* and other confounding factors that may mitigate gut resistance, interventions will be evidence-directed. These interventions would be aimed at regulating identified drivers of antibiotic resistance, thus reducing reported morbidity and mortality and other negative impacts of human, environmental, and economic consequences of increasing antibiotic resistance [64–66].

From the knowledge of this field, coupled with the findings of this review that revealed the enrichment of ARGs with increases in the abundance of specific bacteria taxa, it is important to consider the modification of the gut microbiota, such as the use of specific probiotic and prebiotic therapies as future strategies in addressing antibiotic-resistance in humans. Probiotics have been shown to decrease the abundance of antibiotic-resistant bacteria through competitive exclusion and the production of antibacterial compounds, improve immunity through immune system modulation, and restore a balanced microbiota after gut dysbiosis following antibiotic treatment [67–71]. Probiotic research is ongoing, particularly knowledge of its use as a complementary therapy. The efficacy has been shown to depend on various factors, including individual variations in the microbiome of each person, the specific probiotic strain(s) involved, as well as the human health status and their individual characteristics [72].

4.1. Strengths and Limitations of the Study

This study unveiled the potential association between the bacteria microbiota and the gut resistome by looking at studies that employed metagenomic sequencing, offering a more in-depth perspective of the microbiota and resistome compared to conventional culture-based approaches or other molecular-based techniques, a significant strength of this review.

Despite this strength, the observations here should be taken in the context of the limitation that no cut-off restrictions were made regarding the number of study participants for the eligibility of the studies. An appropriate sample size increases precision and higher power to determine minor effects. Also, studies eligible for inclusion were limited and from a limited number of countries despite the comprehensive search, which may limit the generalizability of the findings. This review was not registered with PROSPERO or another public database. This is because the review is part of a larger research project initially designed to address broader objectives within a specific framework. In due course, the observations will prompt a need for public dissemination. Although not registered, a rigorous systematic review procedure and principles were followed.

4.2. Conclusions and Future Directives

In conclusion, the bacteria gut microbiota is associated with the gut resistome. Lower diversity in the microbiota is likely a significant contributor to higher gut resistome abundance. Also, a higher relative abundance of Proteobacteria, especially members within the Enterobacteriaceae family, particularly *E. coli*, can significantly contribute to resistome enrichment. Despite high heterogeneity and low granularity in the data, there was evidence that *Bifidobacterium* was the most abundant genera in healthy participants carrying *E. coli* ARGs. In infants, this was followed by *Escherichia* or other Proteobacteria genera, as opposed to genera belonging to Actinobacteria or Bacteroidetes in adults. There is a dearth

of studies that use metagenomic sequencing technologies to explore human gut microbiota and resistome, the outcome of which could be used to identify drivers in the spread and dissemination of antibiotic resistance.

A critical component of the solution to antibiotic resistance is high-quality and innovative research that produces large and well-characterized prospective data sets. Such data are essential in developing evidence-driven policy briefs that strengthen regulations and foster effective antibiotic resistance stewardship programs. Therefore, more studies should be undertaken in this area. Exploring publicly available gut metagenomic datasets, which were not initially set to examine the resistome, also holds the potential to yield valuable insights into the gut resistome. Such analyses would contribute to narrowing the substantial research gap, complementing our understanding of the microbiota influence on the resistome, and assist in identifying other microbiome drivers associated with gut ARG enrichment. Additionally, research could also focus on investigating the fundamental mechanisms that link gut microbiota diversity to the abundance of antibiotic resistance genes. This could involve exploring metabolic pathways or microbial interactions that facilitate the transfer of resistance genes, with particular attention paid to critical periods in early life when the gut microbiota is most susceptible to resistome enrichment.

Supplementary Materials: The supplementary materials can be downloaded at https://www.mdpi.com/article/10.3390/microbiolres15030107/s1.

Author Contributions: Conceptualization, J.F. and P.O.B.; methodology J.F. and P.O.B.; validation, J.F., M.R., L.G.M.-R. and P.O.B.; formal analysis, J.F., M.R., L.G.M.-R. and P.O.B.; writing—original draft preparation, F.J; writing—review and editing, J.F., M.R., L.G.M.-R. and P.O.B.; supervision P.O.B.; project administration, L.G.M.-R. and P.O.B.; funding acquisition, P.O.B. All authors have read and agreed to the published version of the manuscript.

Funding: The research reported in this study was supported by the South African Medical Research council (SAMRC). Support for J.F. was through the division of the Research Capacity Development under the SAMRC Extramural Postdoctoral Programme. L.G.M-R. was supported by the Future Professors Programme and the University of Venda Capacity Development Programme. The content hereof is the sole responsibility of the authors and do not necessarily represent the official views of the funders.

Data Availability Statement: All data are contained within the article and the Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

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