FACULDADE DE MEDICINA VETERINÁRIA

DIETARY HABITS AND ANTIMICROBIAL RESISTANCE: A METAGENOMIC CHARACTERIZATION OF HUMAN RESISTOMES ACROSS EUROPE

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UNIVERSIDADE DE LISBOA

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Hábitos Alimentares e Resistência aos Antimicrobianos: Uma Caracterização Metagenómica dos Resistomas Humanos na Europa

Resumo

A resistência aos antimicrobianos (RAM) é um problema crescente a nível mundial, exigindo uma análise aprofundada dos seus determinantes nas populações humanas. Este estudo exploratório caracteriza o resistoma humano em populações europeias, com base na análise de 932 conjuntos de dados metagenómicos obtidos através do American Gut Project. Foram examinadas possíveis correlações entre regiões geográficas, frequência de genes de RAM e padrões alimentares, classificados em consumo de carne vermelha e de aves (RPMC), ausência de carne vermelha (NRMC) e dietas vegetarianas ou veganas (NMC).

Foi utilizada uma metodologia bioinformática padronizada para detecção de genes de RAM com base na base de dados PanRes e na ferramenta KMA. A classificação taxonómica das bactérias foi realizada com o Kraken. As abundâncias foram normalizadas por meio da transformação Additive Log-Ratio (ALR), a fim de minimizar os enviesamentos associados à natureza composicional dos dados metagenómicos.

Os resultados indicam pequenas diferenças na abundância relativa de genes de RAM entre tipos de dieta, com valores mais elevados observados no grupo que segue uma dieta omnívora com consumo de carne vermelha e de aves (RPMC). Entre os diferentes países, também se observou variação na abundância relativa de genes de RAM, com valores mais elevados registados na Alemanha, Bélgica, Países Baixos e Dinamarca. No entanto, estes padrões descritivos devem ser interpretados com cautela, devido ao número limitado de amostras positivas para RAM e à distribuição desigual entre os grupos analisados.

Embora os resultados sejam essencialmente descritivos, apontam para a potencial importância de factores dietéticos e geográficos na vigilância da RAM. São necessários estudos adicionais com amostras mais equilibradas e metadados ambientais e dietéticos detalhados. Este estudo destaca ainda a relevância da abordagem *One Health* na resposta integrada ao desafio da resistência aos antimicrobianos.

Palavras-chave: Resistência Antimicrobiana, Resistoma Humano, Hábitos Alimentares, Metagenómica

Dietary Habits and Antimicrobial Resistance: A Metagenomic Characterization of Human Resistomes Across Europe

Abstract

Antimicrobial resistance (AMR) is a growing worldwide problem that calls for a detailed study of its determinants in human populations. This initial study outlines the human resistome found among European populations based on a study of 932 metagenomic data sets obtained through the American Gut Project. This study examines possible relationships between the abundance of AMR genes and geographic regions as well as diet patterns classified as red and poultry meat consumption (RPMC), non-red meat consumption (NRMC), and vegetarian or vegan (NMC).

A standard bioinformatics workflow was used, including the PanRes Database and KMA to discover antimicrobial resistance (AMR) genes, and Kraken for taxonomic bacterial classification. Abundances were normalized by using Additive Log-Ratio (ALR), due to the compositional nature of the data.

The results indicate small differences in the relative abundance of AMR genes between dietary groups, with numerically higher values observed in the Red and Poultry Meat Consumption (RPMC) group. Across countries, variation in AMR gene abundance was also observed, with Germany, Belgium, Netherlands, and Denmark presenting higher relative values than other regions. However, these descriptive patterns must be interpreted with caution due to the limited number of AMR-positive samples and the uneven distribution across groups.

While the results are mainly descriptive, they highlight the potential importance of dietary and geographical factors in AMR surveillance, which supports the relevance of a One Health approach in addressing AMR from an integrated perspective. It is however essential to perform further studies using large and well-designed datasets in order to confirm the findings.

Key-Words: Antimicrobial Resistance, Human Resistome, Dietary Habits, Metagenomics

Resumo Alargado

Hábitos Alimentares e Resistência aos Antimicrobianos: Uma Caracterização Metagenómica dos Resistomas Humanos na Europa

A resistência aos antimicrobianos (RAM) constitui um dos maiores desafios de saúde pública do século XXI, que ameaça as práticas clínicas modernas e as infraestruturas de saúde pública em todo o mundo. Apesar da quantidade substancial de estudos realizados sobre os antimicrobianos e o fenómeno correspondente da resistência, ainda existem importantes lacunas de conhecimento sobre os mecanismos através dos quais a dieta e a geografia podem influenciar padrões de resistência. Este estudo visa preencher esta lacuna científica ao fornecer uma descrição metagenómica dos resistomas humanos em diferentes populações europeias, explorando se e como a dieta e a geografia podem influenciar a frequência e abundância dos genes de RAM.

O principal objetivo deste estudo foi realizar comparações exploratórias sobre até que ponto a dieta, mais precisamente, o consumo de carne vermelha e de aves (RPMC), a evitação da carne vermelha (NRMC) e a adoção de dietas vegetarianas ou veganas (NMC), pode influenciar tanto a abundância como a diversidade dos genes de resistência antimicrobiana na microbiota intestinal humana.

Para cumprir este objetivo, o estudo analisou 932 amostras metagenómicas obtidas do intestino humano pelo projeto American Gut Project (AGP). As amostras foram selecionadas intencionalmente com base na correspondência geográfica com os países europeus participantes no projeto EFFORT. As amostras foram classificadas em diferentes padrões dietéticos (RPMC, NRMC, NMC) com base em metadados dietéticos disponíveis. Utilizou-se uma estratégia rigorosa de controlo de qualidade (QC) e pré-processamento das amostras com o FoodQCpipeline e bbtools, para remover leituras de baixa qualidade, adaptadores e contaminantes, assegurando leituras limpas e de alta qualidade.

A deteção e quantificação dos genes de resistência antimicrobiana recorreu a ferramentas bioinformáticas, incluindo a base de dados PanRes, uma base altamente curada para genes de RAM e o K-mer Alignment (KMA), um software computacionalmente eficiente e altamente eficaz para estudos metagenómicos. O KMA permitiu o mapeamento exato de leituras curtas de metagenomas para a base de dados PanRes, identificando clusters de genes de resistência. As abundâncias relativas dos clusters de genes foram normalizadas usando transformações aditivas log-rácio (ALR), que ajustam a variação da composição entre as amostras devido a desequilíbrios nas estruturas das comunidades microbianas.

As contagens bacterianas foram obtidas através do Kraken, proporcionando uma visão geral da composição bacteriana ao longo do conjunto de dados. Embora não tenha sido realizado um perfil taxonómico completo, estas contagens serviram de base para os processos subsequentes de normalização, permitindo comparações mais precisas das abundâncias dos genes de resistência aos antimicrobianos entre os grupos dietéticos e geográficos. A análise descritiva identificou também padrões preliminares de relacões entre dieta e geografia com disparidades na composição dos resistomas humanos.

Dietas classificadas como omnívoras (RPMC) apresentaram valores numericamente mais elevados de abundância relativa de genes de RAM em comparação com dietas vegetarianas ou com restrição de carne (NMC e NRMC). No entanto, estas diferenças devem ser interpretadas com cuidado, dado o número reduzido de amostras disponíveis nos grupos não carnívoros. A abundância relativa de genes de RAM também variou entre países, com valores mais elevados observados na Dinamarca, Países Baixos, Alemanha e Bélgica, e mais baixos em países como Itália, Bulgária e Espanha. Estas variações são apresentadas de forma descritiva e não devem ser interpretadas como reflexo direto de políticas nacionais ou outros determinantes contextuais, dada a natureza exploratória do estudo e a distribuição desigual das amostras entre países.

A resistência a tetraciclinas, beta-lactâmicos e macrólidos foi encontrada em diferentes dietas e países, refletindo presumivelmente o uso generalizado destes antibióticos na medicina humana e veterinária em toda a Europa, apontando para a necessidade de programas específicos de gestão antimicrobiana.

Estes resultados exigem uma consideração meticulosa das limitações subjacentes, incluindo um tamanho relativamente modesto das amostras, distribuições não uniformes das amostras entre países e padrões alimentares, e falta de informação completa sobre o uso de antibióticos nas populações estudadas. Além disso, as bases de dados publicamente disponíveis podem carecer de variáveis contextuais importantes, como os hábitos de aquisição alimentar, estatuto socioeconómico e acesso a cuidados médicos, que podem afetar a composição do resistoma no trato gastrointestinal.

Apesar destas limitações, este estudo exploratório inicial fornece as primeiras indicações de possíveis associações entre hábitos dietéticos, origens geográficas e prevalência de RAM nas populações humanas da Europa. Destaca a complexidade das vias de transmissão da RAM e confirma a necessidade de uma abordagem

multidisciplinar "Uma Só Saúde" (One Health) na vigilância e controlo da RAM. Além disso, sugere a importância dos componentes dietéticos nas estratégias de saúde pública para abordar a RAM, indicando o caminho para futuras investigações através de intervenções dietéticas e alterações políticas no uso agrícola de antibióticos.

Estudos futuros devem procurar expandir a dimensão populacional da amostra, alcançar uma distribuição ótima das amostras e utilizar documentação detalhada do uso de antibióticos. Ensaios metodologicamente robustos de intervenções dietéticas e estudos longitudinais baseados em metagenómica funcional podem testar adequadamente e esclarecer estas observações preliminares, estabelecendo com maior precisão se os genes de resistência identificados são funcionalmente expressos e clinicamente relevantes para o avanço da compreensão da dinâmica de transmissão da RAM.

Em conclusão, esta investigação fornece a plataforma de base para explorar a possível interação entre hábitos dietéticos, fatores geográficos e a disseminação dos genes de RAM nos microbiomas intestinais humanos na Europa. Destaca a necessidade essencial de programas abrangentes de monitorização da RAM e estratégias integradas de gestão informadas pelo conceito Uma Só Saúde, para combater o problema global da resistência antimicrobiana de forma eficaz.

Palavras-chave: Resistência Antimicrobiana, Resistoma Humano, Hábitos Alimentares, Metagenómica

Table of Contents

List of Figures

Abreviaturas e siglas

AB - Antibiotic

ABs - Antibiotics

ALR - Additive Log-Ratio

AMR - Antimicrobial Resistance

AMRB - Antimicrobial Resistance bacteria

ARG - Antimicrobial Resistance Genes

BE - Belgium

BG - Bulgaria

DE - Germany

DK - Denmark

ENA - European Nucleotide Archive

ES - Spain

EU - European Union

FAO - Food and Agriculture Organization

FR - France

GLASS - Global Antimicrobial Resistance and Use Surveillance System

HGT - Horizontal Gene Transfer

HMP - Human Microbiome Project

IT - Italy

KMA - K-mer Alignment

NMC - No Meat Consumption

NL - Netherlands

NRMC - No Red Meat Consumption

PL - Poland

RPMC - Red and Poultry Meat Consumption

WHO - World Health Organization

WOAH - World Organisation for Animal Health

1. Activities during the Master's Internship

Before starting the internship in Denmark, I undertook a preparatory training period from September to December 2023 at the Faculty of Veterinary Medicine, University of Lisbon, under the supervision of Prof. Telmo Nunes. This preliminary internship focused on veterinary epidemiology and provided a solid foundation in data handling, statistical analysis, and the use of R for data science applications. Working with various datasets, I developed essential skills in data transformation, visualization, and critical thinking, which proved crucial for the subsequent research work. This experience also enabled skills such as autonomy, teamwork, and adaptability, competences that were important during the main internship period.

My curricular internship took place at the National Food Institute of Denmark, where I worked on bioinformatics, metagenomic analysis, and AMR research. The internship initially focused on evaluating the transmission of antimicrobial resistance between food-producing animals and humans by investigating whether AMR genes commonly found in animals were also present in human gut microbiomes. However, due to the limited number of relevant samples, the scope was adjusted to the analysis of AMR gene abundance in human populations with different dietary habits across Europe, leading to my master's thesis, titled "Dietary Habits and Antimicrobial Resistance: A Metagenomic Characterization of Human Resistomes Across Europe."

Throughout this period, I was responsible for retrieving and processing public metagenomic datasets from healthy human populations, ensuring proper metadata extraction and sample structuring for analysis. I applied bioinformatics and computational methods to assess the presence and distribution of AMR genes across different dietary groups and countries. Using tools such as Kraken for taxonomic classification and KMA and Bowtie2 for AMR gene identification, I characterized the resistome profiles of individuals with different dietary habits.

To further analyse patterns in AMR gene abundance, I implemented data normalization techniques, conducted descriptive statistical analyses, and explored multivariate approaches to identify potential patterns. Additionally, I applied machine learning algorithms in R and Python to explore potential predictive relationships between dietary habits and AMR transmission dynamics.

While the study's initial goal was to examine AMR gene overlap between human and animal microbiomes, the revised focus allowed for a targeted investigation into how dietary choices may influence AMR exposure in humans.

During the internship, I engaged in weekly follow-up meetings with my supervisors, actively discussing research progress and challenges. I collaborated with a multidisciplinary research team, gaining experience in scientific communication, problem-solving, and independent research development. At the end of the internship, I presented my findings to the host research group, strengthening my oral presentation and scientific reporting skills.

This experience significantly expanded my expertise in bioinformatics, data science, and antimicrobial resistance research, enhancing my ability to handle large-scale metagenomic data, apply computational analysis tools, and critically interpret AMR patterns. It also underscored the importance of adaptability in research, as shifting objectives based on data availability was essential in refining the study's scope. Ultimately, the internship provided valuable hands-on experience that has shaped my approach to scientific research and strengthened my ability to contribute to the field of AMR surveillance and microbiome studies.

2. Main objectives and study design

The main goal of this research was to investigate AMR in human populations after the inspiration provided by the EFFORT project (2017), which investigated the resistance of animals in certain countries of Europe. Whereas EFFORT aimed at cataloguing resistance patterns in animal populations, this research broadens its scope to the human resistome, investigating metagenomic data sets from the same nations to determine if there are variations in the resistance profiles.

Among the main objectives of this study was to see geographically how AMR patterns differ and if they align with dietary patterns. By combining metagenomic data and dietary data, the study sought to identify if dietary patterns, red meat, no red meat, and vegetarian diets are linked with resistome structures. Identifying these associations may give insight into environmental and lifestyle determinants of antimicrobial resistance in human populations.

Aside from the detection and characterization of AMR gene clusters from human metagenomic samples, a secondary goal of this research was to develop a robust and reproducible methodological pipeline for detecting AMR gene clusters using publicly available data. This will make the results reliable and open to follow-up studies on the human resistome.

3. Literature Review - Antimicrobial Resistance: A Looming Global Crisis

3.1. Emergence and Evolution of Antimicrobial Resistance

Antimicrobials are one of the cornerstones of modern medicine. They are used to prevent and treat infectious diseases in humans, animals and plants (Murray et al. 2022). So, if we return to the origin of the use of antimicrobial substances in medicine, it goes as far back as the ancient Egypt, where mouldy bread and honey were used on infected wounds(Kopp et al. 2003). In the 19th century, Joseph Lister and Louis Pasteur observed mould that could inhibit the growth of bacteria, with Lister successfully treating injuries using *Penicillium glaucum* in 1871. In 1889, Jean Paul Vuillemin defined 'antibiosis' as any biological relationship in which “one living organism kills another to ensure its own existence”. In 1909, Paul Ehrlich discovered arsphenamine for the treatment of syphilis(Gelmo 1908).

The search for substances to combat infections has a long and complex history, with each discovery building upon previous findings. A breakthrough occurred in 1928, when Alexander Fleming accidentally discovered the first true antibiotic, penicillin, after observing that a fungus inhibited the growth of *Staphylococcus aureus* colonies (Fleming 1929).In the following decade, Gerhard Domagk identified the antibacterial properties of sulphanilamides, which were later marketed as Prontosil® in 1935 and played a crucial role during World War II (Lewis 2013). Although the potential of penicillin was recognized early, it was not produced on an industrial scale until 1940.

Since then, antibiotics (AB) have become the most significant class of pharmaceuticals and one of the most influential medical inventions and are a boon to human society in the fight against bacterial infections, saving millions of lives (Cunha et al. 2019). However, the activity of AB is challenged by the ability of the bacteria to develop resistance against them, thus compromising their efficacy (Gajdács and Albericio 2019). By the late 1960s, the emergence of antibiotic-resistant pathogens was already evident, with penicillin-resistant strains having emerged has early has the 1940s. (Spellberg et al. 2008). In the decades that followed, particularly during the so-called “golden era” of antibiotic discovery (1940s–1960s), the pharmaceutical industry developed a steady amount of new antibiotics, which helped mask the growing threat of resistance. This abundance of new drugs created a false sense of security, leading to a lack of sustained investment in resistance monitoring and stewardship strategies (Anderson et al. 2023). As a result, the urgency to address antimicrobial resistance diminished, allowing it to become a silent but escalating global health crisis.

The rapid dissemination of antibiotic resistance in pathogens has rendered once highly effective antibiotics obsolete in recent decades (Lee Ventola 2015). The struggle against antibiotic resistance underscores the ongoing need for adaptive research strategies in managing infectious diseases (Sengupta et al. 2013). The limited introduction of new antibiotic classes for medical use, with the last new class (oxazolidinones) introduced in 1990, underscores the urgency of discovering novel antibiotics with prolonged efficacy against life-threatening infections (Ragnar Norrby et al. 2005).

As we confront the depletion of antibiotic reserves, concerns grow about the potential regression to a pre-antibiotic era (Brandt et al. 2014). The clinical management of infections by antibiotic-resistant pathogens presents an increasing challenge, demanding for solutions (Brandt et al. 2014).

3.2. Economic and Social Impact on Human Health

On 22 October 2015, the World Health Organization (WHO) has launched the Global Antimicrobial Resistance and Use Surveillance System (GLASS), and global data reveals a long withstanding increase in antimicrobial resistance (AMR), now identified as one of the leading causes of death (Murray et al. 2022).

In 2019, 4.95 million deaths were attributed to bacterial AMR globally, with 1.27 million specifically linked to bacterial resistance (Murray et al. 2022). The United States witnessed more than 2.8 million infections caused by antibiotic-resistant bacteria (CDC 2019) and the report shows that Western sub-Saharan Africa has the highest death rate due to bacterial AMR, at 27.3 deaths per 100,000 individuals (Murray et al. 2022).

AMR is also a major public health concern in the WHO European Region, with estimates from the European Union/European Economic Area (EU/EEA) alone showing that each year more than 670 000 infections are due to bacteria resistant to antibiotics and approximately 33 000 people die as a direct consequence(ECDC 2022).

The impact of AMR on Human healthcare systems is increasing as the effectiveness of antibiotics against bacterial infections decreases. Treating resistant infections is getting more challenging, and as a result, patients need longer hospitalizations and more isolation beds. All of this has a cost, directly increasing with the raise of the resistance levels, and walking side by side with the prices of the treatments, that will become more expensive both for patients and hospitals. Some studies point that AMR can cost from $300 billion to $2.9 trillion worldwide, by 2050 (Watkins and Bonomo 2016; World Bank Group 2017).

There are also secondary effects of AMR on non-infectious medical conditions. For example, surgeries that usually require the use of antibiotics to decrease the risk of post-surgery infections may become compromised. This implies that medical conditions currently treatable through procedures such as surgery, transplants, or dialysis may become untreatable (Santoro-Lopes and De Gouvêa 2014; Naylor et al. 2018). Similarly, AMR is expected to influence the path of diseases like cancer. The immune system of cancer patients is compromised by chemotherapy, making them vulnerable to infections. If this cannot be addressed with effective antimicrobials, the risks associated with chemotherapy may increase (Dadgostar 2019).

Beyond the substantial expenses associated with antibiotic research and development, the accelerated evolution of AMR has led to low investment returns for the pharmaceutical R&D industry. Because of this, numerous pharmaceutical companies have already abandoned antibiotic research and the development of new antibiotics (Uddin et al. 2021).

The impact of AMR does not only apply to human lives, but also to livestock. Just like in human medicine, animal treatment will be less effective leading to more severe infections(Dadgostar 2019), and increasing mortality and morbidity (Hao et al. 2014). As a consequence, animal production might decrease and products such as meat, milk and eggs, protein sources with rising demand worldwide (Van Boeckel et al. 2015), may become less available and less affordable (World Bank Group 2017; Dadgostar 2019). The estimates predict that if the patterns in AMR do not change, 11% of livestock production will decrease by 2050 (World Bank Group 2017), which will also come with animpact in income generation (Dadgostar 2019).

The AMR crisis poses a severe challenge to both human and animal lives, as well as to the global economy.

3.3. Public Health Implications of Human-Animal AMR Transmission

The spread of antibiotic resistance through human contact with animals poses a significant threat to public health. The use of antibiotics in livestock production and veterinary medicine has accelerated the emergence of antibiotic resistance in zoonotic bacteria, by promoting the horizontal transfer of resistance genes across different bacterial species. The food supply chain, environmental contamination, or animal husbandry can all serve as a gateway to the zoonotic transmission of AMR, and lead to gradual spread of resistance across different ecosystems (Jin et al. 2023).

Interconnectedness among resistant bacteria from animals and humans has been demonstrated in several epidemiological studies. The coinciding antimicrobial

resistance genes (ARGs) in human clinical isolates and those found in bacteria from farm animals suggests the movement of resistance determinants across species. This is most problematic in the context of long-term antibiotic application in animal husbandry, or the use in animals of antibiotics critical for human treatment, which may promote the emergence of multidrug-resistant bacterial strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) and ESBL-producing *Escherichia coli* are examples of resistant bacteria with some degree of zoonotic transmission(Köck et al. 2013; Iramiot et al. 2020).

Besides the possible direct health effects, transmission of AMR at the human-animal interface also impacts public health policy. The zoonotic transmission of resistant bacterial strains into human populations may lead to regional and global outbreaks, thus underlining the importance of human-animal interaction in shaping AMR epidemiology. (WHO 2024).

The environment serves as a reservoir and vector for resistant bacteria. Both antimicrobial drugs and resistant microbes can move into soil and water systems through agricultural run-off and inadequate waste management. Wildlife can subsequently acquire and spread these resistant strains through ecosystems, possibly introducing them to human populations. Research has indicated that wildlife species, though often recipients of AMR, are vectors that can transmit resistance through numerous environmental pathways (Olaru et al. 2023).

The consumption of animal food products infected with resistant bacteria is a significant pathway of AMR spread to humans. Antimicrobial administration in food-producing animals can select for resistant bacterial populations, which can be passed on to humans through the food chain. A review has demonstrated the potential of food animals to act as reservoirs of antimicrobial resistance genes encoding for resistance against antibiotics of clinical importance for human treatment (Vezeau and Kahn 2024; Mcewen and Fedorka-Cray 2002).

Lastly, understanding the transmission dynamics of AMR between animals, the environment and humans is essential to adequately quantify the public health impact of resistance and predict future trends. Extensive scientific evidence underlines the importance of an integrated, holistic AMR research strategy, acknowledging the dynamic interface of animal, environmental and human microbial populations and the complex transmission pathways of resistance (McEwen and Collignon 2018).

3.4. AMR: A threat to Food Safety

The food chain has a critical role in AMR transmission, due to its action in connecting animals, the environment, and humans. The increase in consumption of meat

has intensified this link, due to the increased use of antibiotics in livestock production and its contribution to the development of AMR (Ruiz and Alvarez-Ordóñez 2017). Globally, livestock raised for food accounts for 73% of antibiotic usage during production (Van Boeckel et al. 2015; Van Boeckel et al. 2019). This usage in livestock production surpasses that in vegetable crop farming (Jans et al. 2018).

In addition to various foods such as vegetables, fruits, dairy products, and eggs (Acar and Moulin 2006), it is estimated that approximately 50% of meat and seafood products are contaminated with bacteria (Jans et al. 2018). Therefore, proper surveillance of AMR bacteria from farm-to-fork in the food chain is critical to control the zoonotic spread of resistance (EFSA 2014).

As said earlier, the environment is also involved in AMR transmission. The ABs that are used in animals and humans are excreted and their residues may remain active in waste, eventually re-entering the food chain via the application of manure or wastewater in crops (Thanner et al. 2016).

The significance of food as a pathway for the spread of AMR is not always adequately recognised (EFSA 2008). The primary concerns in this area involve food spoilage and consumption of raw or undercooked products, which serve as pathways for antimicrobial-resistant bacteria (AMRB) to enter the food supply chain (Bohaychuk et al. 2009; Hansen et al. 2010; Losio et al. 2015; Di Ciccio 2021). There are different routes via which AMR can be transferred to humans through the food chain, whether it is indirectly, through food consumption or directly, through contact with infected animals or their biological fluids, for example during slaughter: blood, urine, feces, saliva and semen (Chang et al. 2015).

The relevance of this transmission pathway is being increasingly recognised, adding importance to the epidemiology of AMR in livestock, which has been a growing area of research (Hedman et al. 2020). By that research, it is observable that the AMR prevalence in broilers and pigs is increasing in most countries of Europe (Hesp et al. 2019), justifying the urge to establish a proper AMR surveillance system for livestock to comprehend the dimension of this problem (Mader et al. 2022). However, data from the European Food Safety Authority (EFSA 2024) highlight that AMR trends are not universally increasing, with variations observed between countries, bacterial species, and antimicrobial classes, and some instances showing stable or decreasing resistance patterns.

3.5. The One Health Perspective of AMR

Bacteria gain resistance through natural selection, for example by acquiring resistance genes that confer them an evolutionary advantage. Such genes can spread across different bacterial species inhabiting in different reservoirs (Bürgmann et al. 2018; Kimani et al. 2019). AMR emerges as a threat affecting the three One Health pillars: humans, animals, and the environment. Thus, a One Health surveillance system is essential to understand the AMR epidemiological challenge, and to uncover the links between AMR and an irresponsible use of antimicrobials in the diverse sectors (JIM O'NEIL 2016; Collignon et al. 2018), inadequate infection control, and the contamination of the environment (Bürgmann et al. 2018; Kimani et al. 2019)

Interdisciplinary and global measures are crucial for controlling AMR (Collignon and McEwen 2019). That said, organizations such as the World Health Organization (WHO), the World Organisation for Animal Health (WOAH, formerly OIE), and the Food and Agriculture Organization of the United Nations (FAO) have long collaborated on the Global Action Plan on Antimicrobial Resistance (WHO 2017). More recently, this collaboration has evolved into the Quadripartite Alliance, now including the United Nations Environment Programme (UNEP), which coordinates global action on AMR under the One Health approach, addressing the interconnected risks across human, animal, plant, and environmental health. This plan focuses on surveillance and research, guiding countries in the implementation of surveillance and control of antimicrobial use in both humans and animals. A better understanding is obtained from this action plan, facilitating the study of AMR spread and the interventions that are needed across all sectors (Queenan et al. 2016; Magouras et al. 2017).

In response to this multi-layered challenge, the One Health approach presents itself as a crucial strategy, as it not only alerts for the public awareness to the correct use of antimicrobials but also accentuates the importance of an integrated surveillance, research, and joint solutions across human, animal, and environmental health. The goal is to present a united front against AMR, highlighting the link between the three pillars, that share vulnerability and responsibility (Jim O'Neil 2016; Sharma et al. 2018).

3.6. Impact of Dietary Habits on Human Microbiome Resistome

Diet plays a key role in determining the composition of the gut microbiome and, by extension, influences the resistome, the collection of ARGs in microbial populations. Diet influences on the gut microbiome are well established, and specific dietary patterns influence microbial diversity, composition, and function(Conlon and Bird 2015; Rinninella

et al. 2019). This, in turn, can dictate the emergence and spread of AMR genes, rendering diet an essential modifiable component in the battle against AMR.

The gut microbiome is also extremely sensitive to dietary input, and macronutrient composition, for example the consumption of carbohydrates, proteins, and fats, is among the key determinants of microbial community structure (Conlon and Bird 2015; Rinninella et al. 2019). Diets high in fiber and content of complex carbohydrates are favourable for the expansion of health-promoting bacteria such as *Bifidobacterium* and *Lactobacillus*, which are linked with enhanced gut health and reduced relative abundance of antibiotic-resistant genes (Rinninella et al. 2019). On the other hand, high consumption of processed food and straightforward carbohydrates has been associated with low microbial diversity and a rise in potentially pathogenic bacteria, some of which contain resistance genes (Conlon and Bird 2015).

Protein sources also profoundly impact the gut resistome and microbiome. Diets rich in red meat and animal protein have been linked to higher levels of various bacteria known to harbour resistance genes (Rinninella et al. 2019; Tomova et al. 2019; da Silva et al. 2021) Red meat intake, typical in Western diets, has been linked to an expansion of sulfate-reducing bacteria (*Desulfovibrio* spp.) that inflame the gut and potentiate the horizontal gene transfer of resistance genes(Rinninella et al. 2019; da Silva et al. 2021) Furthermore, residues of antimicrobials in conventionally produced animal meat products can lead to resistant strain selection(Milanović et al. 2017; Weinroth et al. 2022a)

Conversely, vegetarian and plant diets enrich *Prevotella*, a genus implicated in carbohydrate metabolism and decrease the relative abundance of putative AMR-harbouring bacteria(Rinninella et al. 2019; Tomova et al. 2019). Vegetarians and vegans are reported to have a lower prevalence of AMR genes in their gut microbiome than omnivores. This can be explained by the avoidance of dietary antibiotics via consumption of animal products(Milanović et al. 2017; Rinninella et al. 2019; da Silva et al. 2021)

The One Health approach supports the hypothesis that eating habits affect personal resistomes, due to the diet's interconnectedness with the environmental and food chain resistomes(Fernández-Trapote et al. 2024). Extensive use of antibiotics in farming practices promotes AMR spread along the food chain, where resistant microbes can be passed on to consumers through contaminated meat, milk, and plant products (Weinroth et al. 2022). This calls for sustainable food policies, including curbing antibiotic usage in farming, encouraging organic farming, and encouraging the consumption of minimally processed, plant-based foods.

Diet is a key determinant of the gut microbiome and resistome. Whereas plant-based, high-fiber diets are associated with increased microbial diversity and low AMR gene loads, high-meat and processed food diets have been associated with high resistance gene loads. Such associations hold out the promise of dietary interventions.

3.7. AMR and Metagenomic Analysis

Metagenomics is a method of DNA survey in the environment that has transformed the study of antimicrobial resistance (AMR) by providing a direct, culture-free approach to the study of microbial communities and their resistance genes. Rather than traditional microbiological techniques that are based on isolating and culturing bacteria under laboratory conditions, metagenomics allows the researcher to sequence the genetic material of complex microbial ecosystems, hence giving a more comprehensive picture of bacterial populations, their functions, as well as the way they harbour resistance (Quince et al. 2017).

One of the highlights of metagenomic techniques is that they can identify both the culturable and non-culturable bacteria, thus scientists can investigate microbial diversity and resistance gene reservoirs in different settings (Berendonk et al. 2015).

Typically, culture-based methods can result in the underestimation of microbial diversity, as a noticeable proportion of the bacteria in human and environmental samples cannot be reproduced in the lab (Lagier et al. 2018). By utilizing a culture-independent approach, metagenomics provides a more accurate assessment of bacterial abundance and antimicrobial resistance gene (ARG) presence/abundance, thus offering a more effective tool for AMR surveillance (van Schaik 2015; Lagier et al. 2018).

Employing metagenomics for AMR surveillance is particularly useful in human health research as it allows the detection of resistance genes, mobile genetic elements, and bacterial taxonomic composition within the same sample. This all-encompassing approach finds out the bacterial resistant strains that might be rising, studies bacterial adaptation, and assesses the impact of selective pressures, for example, the use of ABs and dietary habits on the gut microbiome (Penders et al. 2013). For example, studies on the gut microbiome and antibiotic treatment have found that antibiotic exposure causes a long-term shift in the composition of gut microbes and as a result, AB resistant bacteria become the dominant species (M Pärnänen et al. 2019).

Metagenomic sequencing allows for the detection of horizontal gene transfer (HGT), the main driver of AMR gene dissemination among bacterial species. Consequently, the resistome (the total complement of AMR genes in a microbiome) can undergo rapid evolution through genetic exchange between commensal and pathogenic

bacteria (Forslund et al. 2013). Moreover, metagenomic approaches enable the tracing of resistance gene transmission pathways across ecosystems, linking sources like the human gut microbiome to environmental sinks such as wastewater and soil (Hendriksen et al. 2019). This comprehensive surveillance capability across human, animal, and environmental domains positions metagenomics as a key enabling technology for One Health AMR monitoring frameworks (Martinez et al. 2009).

Notably, large-scale initiatives like the Human Microbiome Project (HMP) and the Metagenomics of the Human Intestinal Tract (MetaHIT) project utilized metagenomics to characterize the human gut microbiome and its associated resistome (Qin et al. 2010), revealing important relationships between host microbiota, diet, and antibiotic exposure.

Taxonomic and functional surveys performed with the use of metagenomics pave the way to trace not only the antibiotic-resistant bacteria but also their ecological roles and interactions in microbial communities (Van Goethem et al. 2019). This function supports the forecasting of resistance trends, the optimization of antibiotic stewardship programs, and the development of the most effective novel antimicrobial strategies. As the discipline continues to develop, the use of metagenomic technology in disease risk surveillance, and what is often interpreted as personalized medicine will likely increase, and thus metagenomics will probably be part of the solution to fight the global problem of antimicrobial resistance. (Franzosa et al. 2018).

4. Methodology

4.1. Data Sources and Selection Criteria

This research used metagenomic data from the American Gut Project (AGP), from samples of 2017 to 2024, explore antimicrobial resistance (AMR) profiles within the human gut microbiome in a comparative parallel to the EFFORT project (2017), which characterized the faecal resistome of food-producing animals in nine European countries. Whereas the EFFORT research gave an understanding of AMR abundance in animal populations, the present study sought to map the human resistome in the same geographical region and examine the association of possible disparities in resistance profiles with dietary lifestyles.

The AGP was chosen as the primary source of human metagenomic samples owing to its large dataset and detailed metadata on participants' lifestyles and diets. AGP contains samples from several countries worldwide, including those investigated within the EFFORT project. With these data in mind, the aim of the study was to determine if dietary habits - no red meat diet, red meat diet, and vegetarian diet - affect the

composition of the human resistome. The combination of metagenomic and dietary data presents important information on possible drivers of resistance in human populations.

By integrating human resistome analysis into its research framework, this study builds upon the EFFORT project by offering a complementary perspective on the expression of AMR in different host populations exposed to shared environmental and dietary conditions. AGP samples were identified through the resource “A curated data resource of 214K metagenomes for characterization of the global antimicrobial resistome” (Martiny et al. 2022), which contains comprehensive information on metagenomic samples from a wide range of global studies. A main criterion for sample selection was the geographic overlap with the countries included in the EFFORT project to ensure consistency in regional representation. The selected human samples were downloaded from the European Nucleotide Archive (ENA) with the project accession number PRJEB11419, while corresponding metadata was obtained from NCBI National Library of Medicine.

These samples were collected using BBL culture swabs (Becton, Dickinson and Company, Sparks, MD) and returned by mail. DNA extraction and sequencing followed the Earth Microbiome Project (EMP) protocols, with the V4 region of the 16S rRNA gene amplified using barcoded primers. Sequencing was performed on various Illumina platforms, using the updated 515f/806rB primer pair. Most sequencing was conducted on an Illumina MiSeq, with some batches processed on an Illumina HiSeq Rapid Run or High-Output platform.

A total of 932 American Gut Project (AGP) human metagenomic samples were chosen considering two important criteria: geographic relevance and the presence of dietary metadata. Among them, 75 were antimicrobial resistant . The choice was limited to those samples submitted between 2017 and 2024 to maintain consistency in sequencing protocols and reduce possible biases related to temporal fluctuation in data collection.

While the animal resistome data from the EFFORT project were not analysed in this study, the countries investigated in EFFORT informed the geographic sampling of AGP samples, in order to allow for a comparative description of AMR profiles within human and food-producing animal populations across the same geographic range.

4.2. Sample Preprocessing and Quality Control

In this study, raw sequencing data obtained from 932 human samples were subject to a rigorous preprocessing and quality control (QC) pipeline to ensure the reliability of downstream analyses.

4.2.1. Quality Control Pipeline

To ensure the reliability of downstream analyses, raw sequencing data were subject to stringent quality control (QC), applying FoodQCpipeline v1. Specifically, this pipeline uses the bbduk2 adapter trimming and quality filtering application from the bbtools suite (Bushnell et al. 2017).

As sequencing errors, contamination, and low-quality data all pose a threat to the integrity of ARG detection, stringent quality control is necessary for any project involving sequencing data. Low-quality sequences, adapter contamination, and the presence of non-target DNA can produce false-positive or false-negative results, compromising the reliability of AMR patterns. To obtain high-quality noise-free sequencing data, and retain only high confidence reads for the AMR gene identification, several QC measures were implemented:

• **Quality filter**: Reads with low-quality bases, particularly at the ends of reads, were filtered out to improve downstream mapping accuracy.

• **Removal of low complexity sequences**: Sequences that have less than a user-defined minimum number of nucleotides were removed from the analysis, as they do not provide enough context to be aligned or assembled.

• **Adapter trimming**: Residual sequencing adapters were detected and removed to eliminate artificial sequence artifacts that may impact metagenomic analysis.

4.2.3. Quality Control Parameters

The following quality parameters were used during preprocessing to ensure the integrity of the human sequencing data:

4.2.3.1 Phred Quality Score Cutoff

Bases with a Phred score below 20 were trimmed from the right end of each read. This ensures that the bases that were left have a confidence level of at least 99%, reducing the sequencing errors in the data. This approach is a standard practice in sequencing projects, as it effectively balances data retention with quality (Rubin et al. 2022). Studies have shown that trimming low-quality bases improves the accuracy of analyses (Overholt et al. 2019).

4.2.3.2 Read Length Threshold

After the trimming process, reads that had less than 50 base pairs were discarded. Short reads generally do not have sufficient information for reliable alignment or assembly, which can introduce noise into the dataset (Mukherjee et al. 2020). By setting this threshold, the dataset's overall quality is maintained, ensuring that only

informative reads are retained for analysis. This step is widely adopted in metagenomic and genomic projects to improve dataset usability and reduce errors in downstream applications (Rajeev et al. 2023).

4.2.3.3 Adapter Trimming

To remove sequencing adapters, the pipeline employs **bbduk2**, which scans the reads using 19-base k-mers. At the ends of the reads, where adapter remnants are more likely and sequences are shorter, the tool switches to shorter k-mers, down to 11 bases, for better sensitivity. Additionally, overlapping regions in paired-end reads are used to enhance the accuracy of adapter trimming. This method minimizes the contamination from adapter sequences that can interfere with the analysis, a key step highlighted by several studies (Ochkalova et al. 2023). By removing such contamination, the integrity of the dataset is preserved.

4.2.3.4 Contaminant Database

The FoodQCpipeline used an internal database of known adapter sequences to identify and then remove contaminants. The use of curated databases for contamination removal is an essential step in this type of project, as pointed out in previous studies (Martin et al. 2018; Rubin et al. 2020). By using such a database, contaminants, including adapter sequences, were efficiently removed, ensuring that only high-quality, biologically relevant sequences were retained for analysis.

4.2.4. Importance of Quality Control for Downstream Analysis

The quality control process was crucial for ensuring the integrity of the raw sequencing data, which is foundational to all downstream analyses. By removing low-quality reads, trimming adapter sequences, and filtering out contaminants, the QC pipeline enhanced the overall reliability of the dataset. This preprocessing ensured that the retained sequences were of high quality, enabling more accurate alignment, assembly, and variant calling.

In the context of this study, where the aim was to characterize AMR patterns in human populations, maintaining high data quality was critical. Without robust quality control, the data could have been influenced by noise, which might have obscured important biological signals or introduced biases into the analysis. Although this study focused on human samples, ensuring consistency and high quality through a uniform QC process facilitates reliable comparisons across datasets, an essential aspect in research on AMR patterns across different regions and populations. Good quality control

also mitigates potential artifacts that could arise from sequencing errors or contamination.

4.3. Antimicrobial Resistance Gene Cluster Identification

4.3.1 Gene Identification Tools

To detect ARGs in the metagenomic dataset, two primary tools were utilized: the PanRes Database and KMA (K-mer alignment). These tools were selected for their ability to efficiently and accurately identify AMR genes from large-scale datasets.

The PanRes Database, developed in 2024, is a curated collection of resistance genes and their variants, providing comprehensive coverage of both well-known and emerging resistance mechanisms.

KMA is a sequence alignment tool optimized for large-scale metagenomic data, using a k-mer-based approach to rapidly align short reads to reference databases such as PanRes. Its speed and accuracy made it the ideal choice for identifying AMR genes in this study.

Detailed descriptions of both tools, including their functionality and role in this study, are provided in the subsequent sections.

4.3.2. PanRes Database – What it is and how it was done

The PanRes Database serves as a powerful resource for AMR gene identification, merging data from multiple existing resistance gene databases. It was developed for redundancy reduction and improved reference library utilization in metagenome analysis by collating vast resistance sequences in a computationally tractable format as a database.

To develop PanRes, the authors assembled genes from several of the commonly used resistance gene databases, consisting of ResFinder, ResFinderFG, CARD, MegaRes, AMRFinderPlus, and ARGANNOT. These repositories offer unique features and resistance gene coverage across a diverse array of bacterial species and mechanisms. Besides these collections, PanRes employs a curated dataset of antimicrobial resistance genes derived from the CsabaPal collection (Daruka et al. 2023), containing cloned and functionally validated ARGs from environmental and clinical samples. This integration was necessary to capture emerging ARGs, especially those conferring resistance to antibiotics that are not yet on the market.

To improve computational efficiency and standardization, all unique sequences in the PanRes Database were assigned a "pan_" identifier (PanRes_genes). A related metadata table (PanRes_data) provides individual records of all genes, their source

database, and any corresponding high-identity gene clusters. For simplicity, gene clusters will be referred to as genes. This organized framework allows researchers to track the lineage of each gene while avoiding duplication of data. The clustering of sequences was carried out by an iterative process using Usearch (Edgar 2010) with a threshold of 90% identity and coverage to maintain stability and accuracy.

PanRes was intended to create a more complete database than previous AMR repositories focused on metagenomes by combining information from several existing AMR databases. Though recently developed and not yet widely used, PanRes fills gaps in existing resources by including genes from various environments and ensuring adequate coverage of new resistance mechanisms. In this study, PanRes was used for the systematic and accurate screening of resistance genes in human metagenomes.

4.3.3. KMA – What it is and why it is used

KMA (K-mer Alignment) is a state-of-the-art sequence alignment software, which provides fast and detailed alignment of sequencing reads of short lengths, especially for large metagenomics projects. In contrast to conventional aligners that struggle with large and often redundant reference databases, KMA uses a k-mer based approach that allows for fast and accurate read alignment to highly similar or overlapping reads (Clausen et al. 2018). This feature is especially useful for mapping ARGs, which tend to have high redundancy due to multiple gene variants within large datasets.

KMA was chosen for this study due to its efficiency in handling large and complex metagenomic datasets and its optimization to handle redundancy effectively, ensuring high-confidence detection of resistance genes across metagenomic samples (Clausen et al. 2018). KMA ensures high-confidence alignments by assessing matches based on k-mer identity and coverage, thus guaranteeing that only the most reliable gene identifications are included in subsequent analyses (Bloemen et al. 2023).

At its core, KMA works by indexing reference databases with k-mers, that are short nucleotide sequences of length k. By aligning sequenced reads to these indexed k-mers, the tool significantly reduces computational load while maintaining high accuracy (Clausen et al. 2018). Additionally, KMA generates consensus sequences, allowing it to identify gene variants and mutations in the aligned sequences, which is particularly valuable for detecting AMR gene variations. This capability ensures a comprehensive identification of resistance genes across metagenomic samples (Clausen et al. 2018; Gand et al. 2024). By enabling a comprehensive and efficient exploration of AMR genes, KMA contributed significantly to the study's scientific rigor.

4.3.4. Gene Identification Process

After the raw data was filtered, the gene identification process was carried out using KMA (K-mer Alignment) against the PanRes Database. The objective of this step was to accurately identify antimicrobial resistance genes clusters from the processed metagenomic samples. Given the large-scale nature of the metagenomic datasets, the steps involved in this process were designed to ensure both the precision of gene identification and the comprehensiveness of the resulting data.

4.3.4.1. Gene Identification and Count Compilation

Each sample was aligned to the PanRes Database using KMA. This k-mer-based alignment approach was crucial for efficient mapping and identifying resistance genes by comparing metagenomic reads against a comprehensive collection of ARGs in the PanRes (Clausen et al. 2018; Herold et al. 2023).. The KMA results for each sample were compiled into a table that listed the counts of each detected AMR gene. These counts indicated the number of times that a specific resistance gene was found within each sample. To ensure accuracy, low-quality or ambiguous matches were excluded based on predefined alignment quality thresholds, allowing only high-confidence gene identifications to be retained for subsequent analysis (Rooney et al. 2022).

4.3.4.2. Handling Replicates

Results from replicated samples were combined to improve the consistency and reliability of the dataset. This step was particularly crucial when multiple samples were obtained from the same individual within the same timeframe. KMA's output for each replicate was combined by summing gene counts for identical AMR genes across the replicates. This merging process ensured that the final table had only one representative result per individual per year, this way reducing the bias due to multiple sampling and offering a more accurate reflection of the gene abundance within each human sample (Clausen et al. 2018).

4.3.4.3. Final Dataset Preparation

The final dataset was a detailed and completed gene count table, with each row representing a unique individual and the columns indicating the identified resistance genes along with their respective counts. This dataset served as the basis for descriptive analyses aimed at characterizing the relative abundance of antimicrobial resistance (AMR) genes across various geographic regions and dietary habits.

4.4. Bacterial Composition Analysis

Accurate bacterial composition assessment is a crucial part of metagenomic studies, especially in analysing antimicrobial resistance patterns. In this study, Kraken was used to classify bacterial taxa to interpret AMR gene patterns in the context of the general microbial community.

4.4.1. Application of Kraken to Analyse Bacterial Composition

Kraken was used to classify bacterial sequences across human samples dataset. Kraken uses a k-mer-based algorithm to assign taxonomic labels to metagenomic reads by mapping them onto a database of known microbial genomes. This k-mer-based use enables it to process massive datasets with a low loss in accuracy in the taxonomic classification (Wood et al. 2014). Kraken provided a comprehensive overview of bacterial composition for each sample by identifying bacterial species to the genus and species levels(Lu et al. 2017).

4.4.2. Bacterial Composition Analysis

The analysis was done to justify the normalization of samples in metagenomic studies. Variation of the bacterial load can introduce bias because samples may be sequenced to different depths. Employing statistical transformations, such as ALR, enables the gene abundance data to be appropriately normalized for changes in bacterial load between samples. ALR is useful for transforming compositional data by underlining a reference component. It serves to stabilize the variances among datasets (Martínez-Álvaro et al. 2024; Yerke et al. 2024), allowing forvalid comparison among samples by reducing the distortions introduced due to differences in sequencing depth or microbial community composition (Gloor et al. 2017).

4.4.3. Importance of Bacterial Counts Toward Analysis of AMR

Analysis of bacterial composition is an indispensable element in the study of AMR, as resistance genes usually are associated with bacterial taxa. In this study, due to ALR normalization, the AMR gene patterns reflected resistance profiles, with minimum impact of variations in microbial composition(Franzosa et al. 2018). This process is critical when realizing an accurate comparison of the AMR profile across human populations in the first place (Ramos et al. 2024). Accurate knowledge of the bacterial counts in each sample was essential to ensure that the AMR gene profiles generated were representative of actual resistance patterns across different human populations, thereby minimizing bias and enhancing the reliability of the results.

4.5. Data Analysis and Characterization

This study adopted a two-step strategy to analyse AMR in human metagenomic samples. It started with a descriptive analysis of the presence of resistance genes and then quantified them using the Additive Log Ratio (ALR) transformation.

4.5.1. Descriptive Analysis of AMR Gene Presence

The dataset contained 932 human metagenomic samples harvested from the American Gut Project (AGP) and collected from six European countries. Out of these, 75 samples displayed antimicrobial resistance genes, which were established by metagenomic sequencing and the following identification pipelines.

The distribution of the AMR-positive samples was assessed among different countries and people with different dietary habits. The presence of the resistance genes was investigated in terms of types of diet, such as red meat consumers, non-red meat consumers, vegetarians and vegans. This preliminary analysis made it possible to identify potential different AMR levels among people with various types of diet and from several European countries.

4.5.2. Characterization of AMR Gene Abundance Using ALR Transformation

The ALR-transformed resistance gene abundances were characterized to explore differences in AMR gene presence across countries and dietary groups. By applying the ALR transformation, the relative abundance of resistance genes was normalized, ensuring that comparisons between countries and types of diet were consistent despite variations in sequencing depth. ALR is widely used in metagenomic studies as it effectively handles compositional data by expressing each variable relative to a fixed denominator, making it particularly useful for comparing relative gene abundances across different sample groups (Gloor et al. 2017; Quinn et al. 2018).

ALR values are often negative due to the mathematical properties of the transformation: when the numerator (AMR gene count) is lower than the denominator (bacterial gene count), the logarithmic ratio yields a value less than zero. The closer the value is to zero, the higher the relative abundance of that gene cluster is in relation to the bacterial background. On the contrary, more negative values indicate lower relative abundance. The descriptive analysis focused on identifying patterns in AMR gene presence, providing an overview of how resistance genes are distributed among different populations.

This approach allowed for a comparative characterization of the human resistomes, highlighting potential patterns in resistance gene abundance across diverse

dietary habits and countries. While no statistical tests were applied due to the limited number of resistant samples, the use of ALR ensured that the relative abundance of AMR genes was appropriately normalized for compositional data, enabling a meaningful descriptive analysis (Martínez-Álvaro et al. 2024). These findings offer valuable insights into possible factors influencing AMR patterns in human populations, providing a foundation for future research with larger datasets.

5. Results

5.1. Descriptive Analysis of Samples

5.1.1. Distribution of samples analysed per Country

The dataset analysed in this study consists of 932 human metagenomic samples collected from nine European countries. As shown in Figure 1, Germany (DE) contributed the highest number of samples (n = 327), representing 35.1% of the total dataset, followed by Belgium (BE) with 154 samples (16.5%) and France (FR) with 151 samples (16.2%). Spain (ES), the Netherlands (NL), and Italy (IT) had moderate representation, with 86 (9.2%), 64 (6.9%), and 54 (5.8%) samples, respectively. Denmark (DK) and Poland (PL) contributed 34 (3.6%) and 51 (5.5%) samples, while Bulgaria (BG) had the lowest representation with 11 samples (1.2%). The variation in sample distribution across countries reflects differences in availability of sequencing data, introducing a sampling bias which should be considered when interpreting geographic patterns in antimicrobial

resistance.

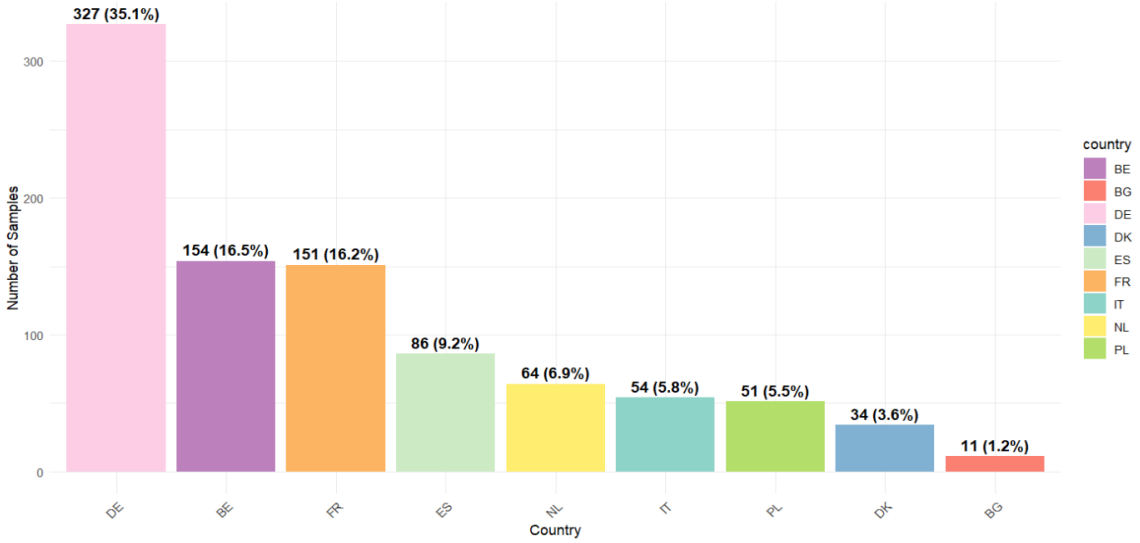

Figure 1. Distribution of human metagenomic samples analysed per country (n=932).

5.1.2. Distribution of dietary habits

Dietary groups include Red and Poultry Meat Consumption (RPMC, omnivorous diet), No Red Meat Consumption (NRMC), and No Meat Consumption (NMC, vegetarian/vegan). Regarding dietary habits the group that had most participants, according to Figure 2, was the RPMC diet (n = 738; 79.2%), indicating a strong predominance of omnivorous dietary patterns. NMC was observed in 110 individuals (11.8%), corresponding to vegetarian or vegan dietary choices, while 64 individuals (6.9%) adhered to a NRMC diet, meaning they consumed poultry and/or fish but avoided red meat. A small proportion of samples (n = 20; 2.1%) lacked dietary metadata and were categorized as Not Available (NA). The observed dietary distribution suggests that meat-inclusive diets are dominant in the dataset, which may have implications for gut microbiota composition and the prevalence of antimicrobial resistance genes.

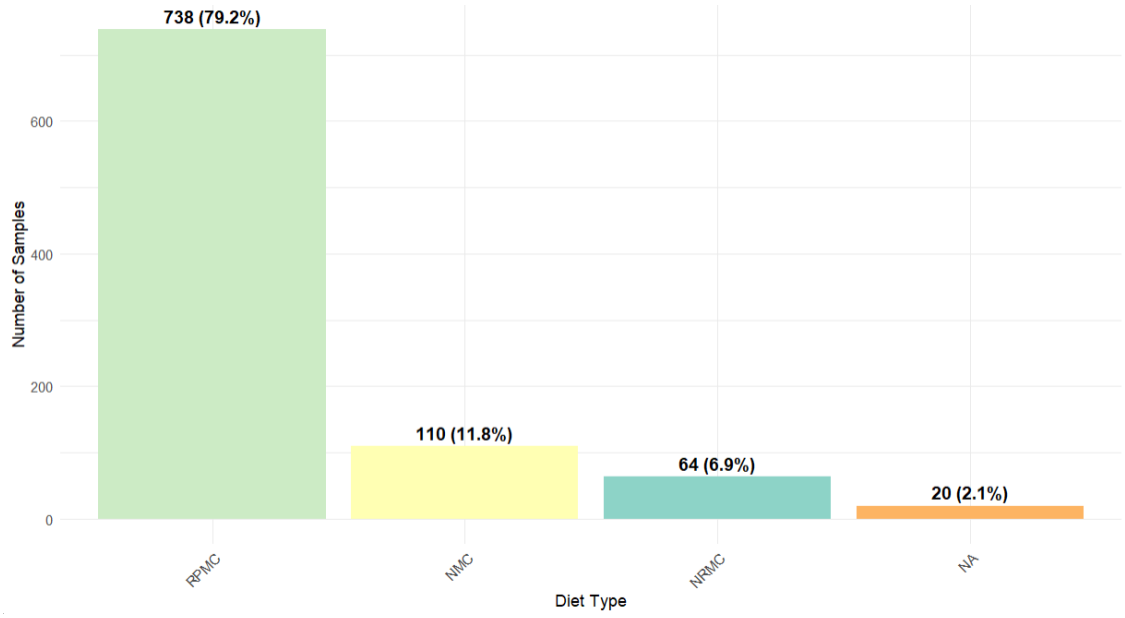

Figure 2. Distribution of analysed samples according to dietary habits (n=932)

5.1.3. Proportion of Samples that present resistance per Country

The proportion of antimicrobial resistance (AMR)-positive samples varied across the nine European countries included in this study. Figure 3 demonstrates that Poland (PL) exhibited the highest proportion of resistant samples, with 13.7% (n = 7/51) of its samples containing detectable AMR genes, followed closely by Italy (IT) at 11.1% (n = 6/54) and France (FR) at 10.6% (n = 16/151). The Netherlands (NL) and Bulgaria (BG) also showed relatively high proportions of resistance, with 9.38% (n = 6/64) and 9.09% (n = 1/11), respectively.

In contrast, Spain (ES) displayed the lowest proportion of AMR-positive samples, with only 1.16% (n = 1/86) containing detectable resistance genes, indicating a relatively low observed prevalence within this limited subset. Other countries, including Denmark (DK) at 5.88% (n = 2/34), Germany (DE) at 7.34% (n = 24/327), and Belgium (BE) at 7.79% (n = 12/154), exhibited moderate levels of resistance. The limited number of samples in the dataset, along with the uneven representation across countries and diets, restricts the robustness of these results.

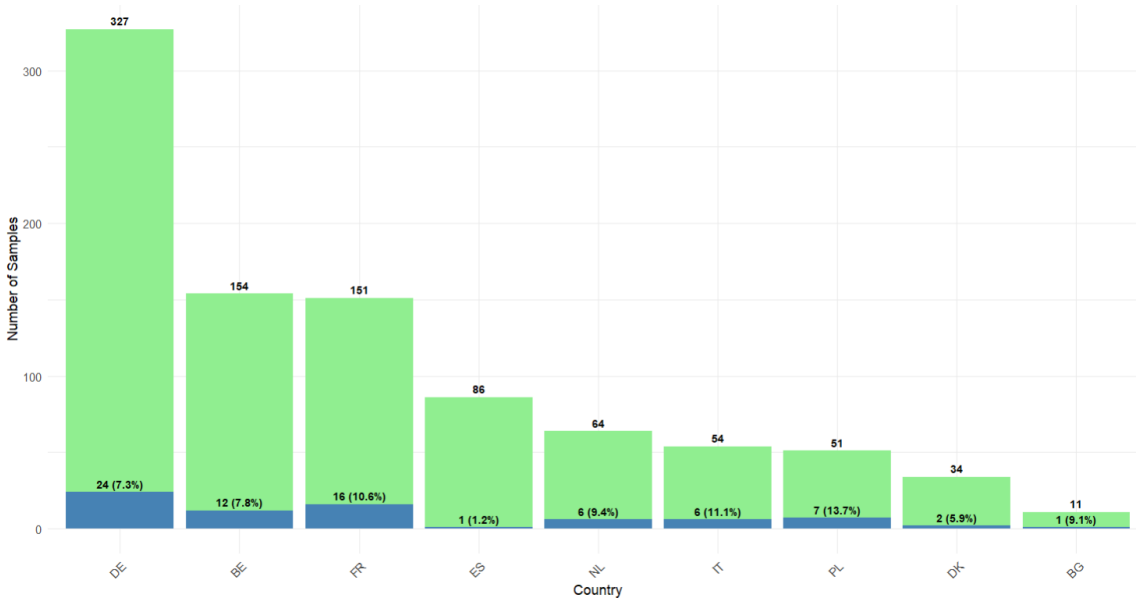

Figure 3. Proportion (%) of AMR-positive samples per country

5.1.4. Proportion of Samples that present resistance per Type of Diet

The proportion of antimicrobial resistance (AMR)-positive samples varied across different dietary groups, highlighting potential associations between dietary habits and the abundance of resistance genes. As demonstrated in Figure 4, the highest proportion of AMR-positive samples was observed in individuals following a Red and Poultry Meat Consumption (RPMC) diet, with 8.81% (n = 65/738) of samples displaying resistance genes. This was followed by individuals who adhered to a No Red Meat Consumption (NRMC) diet, where 7.81% (n = 5/64) of the samples contained AMR genes.

In contrast, individuals categorized as No Meat Consumption (NMC), which includes vegetarians and vegans, exhibited a lower proportion of resistance, with only 3.64% (n = 4/110) of samples testing positive for AMR genes. Despite RPMC individuals showing a higher proportion of AMR-positive samples, the limited sample sizes for NRMC and NMC groups prevent any definitive statistical conclusions about diet-specific AMR abundance.

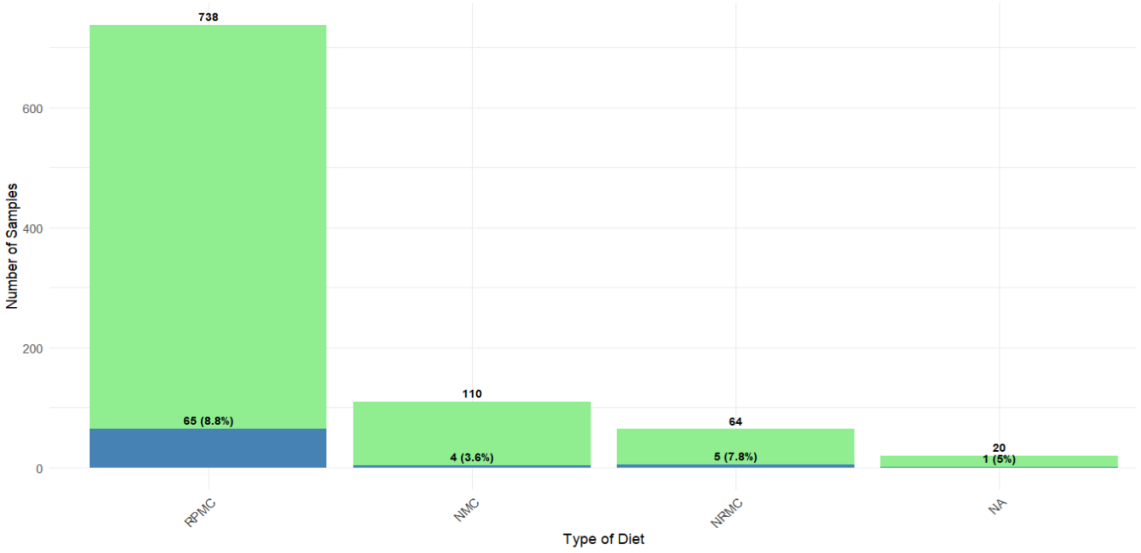

Figure 4. Proportion (%) of AMR-positive samples by dietary type.

5.1.5. Proportion of AMR-Positive Samples per Country and Type of Diet

Following the separate assessments of resistance proportions by dietary group (Section 5.1.4) and by country (Section 5.1.3), this section explores the combined distribution of AMR-positive samples across both dimensions. This integrated view provides a more comprehensive perspective on the relative abundance of resistance in relation to dietary habits within specific national contexts.

Figure 5 represents the Proportion of AMR-Positive Samples per Country and Diet and, demonstrates that among individuals following the Red and Poultry Meat Consumption (RPMC) diet, the highest proportions of AMR-positive samples were observed in France (9.9%) and Poland (9.8%), followed by Bulgaria (9.1%), the Netherlands (7.8%), Belgium (7.8%), and Italy (7.4%). In contrast, Spain and Denmark exhibited lower proportions within this group, with 1.2% and 5.9%, respectively.

In the alternative dietary groups, No Red Meat Consumption (NRMC) and No Meat Consumption (NMC), the proportions of AMR-positive samples were generally lower. For instance, in Germany, while 6.1% of RPMC samples were AMR-positive, only 0.9% were observed among NMC individuals. Similarly, NRMC-associated proportions were 3.7% in Italy, 3.9% in Poland, and 1.6% in the Netherlands. NMC-positive samples were rarely observed, such as in France, where only 0.7% of the total were linked to this group.

This analysis allows for the identification of proportional patterns of resistance when considering both dietary habits and geographical distribution. However, due to the small number of AMR-positive samples in many categories, particularly among non-

RPMC diets and in countries with lower sampling, the interpretation remains purely descriptive and does not allow for inferential conclusions.

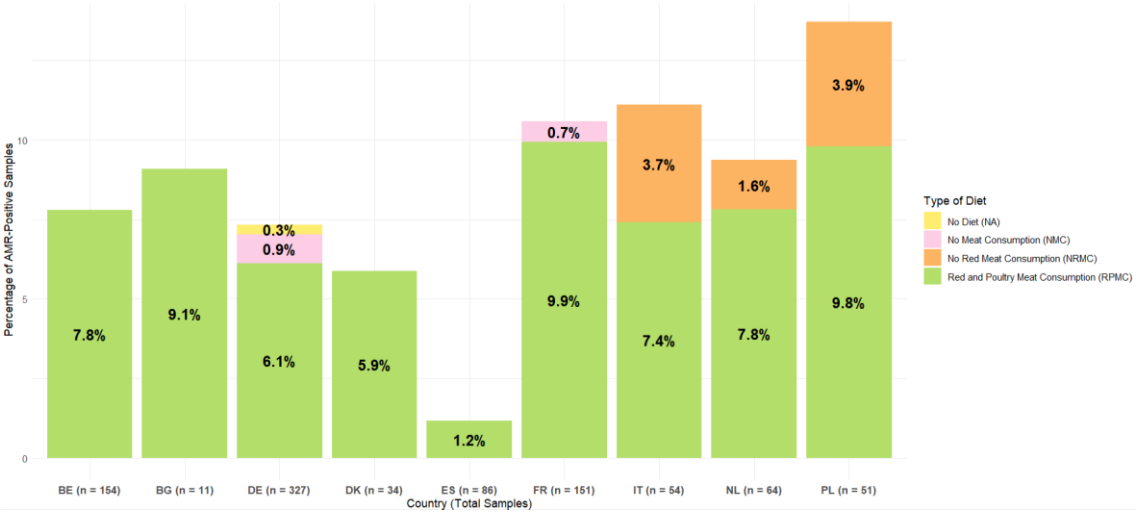

Figure 5. Proportion of AMR-Positive Samples per Country and Type of Diet

5.2. Identification of Antimicrobial Resistance Genes clusters

5.2.1. AMR gene cluster counts per Country

After evaluating sample-level resistance distributions, we identified antimicrobial resistance gene clusters, revealing differences in resistance patterns among countries representing the results in Figure 6.

The distribution of ARGs across countries reveals substantial variations in resistance gene abundance and diversity. Germany exhibited the highest total number of detected AMR genes (n = 3502), followed by France (n = 2670) and Belgium (n = 923). In contrast, Denmark and the Netherlands presented lower AMR gene diversity, with 569 and 274 genes detected, respectively. Due to unequal sample sizes across countries, the total AMR gene counts may be influenced by both sampling depth and underlying geographic factors. Therefore, the results should be interpreted as descriptive rather than comparative.

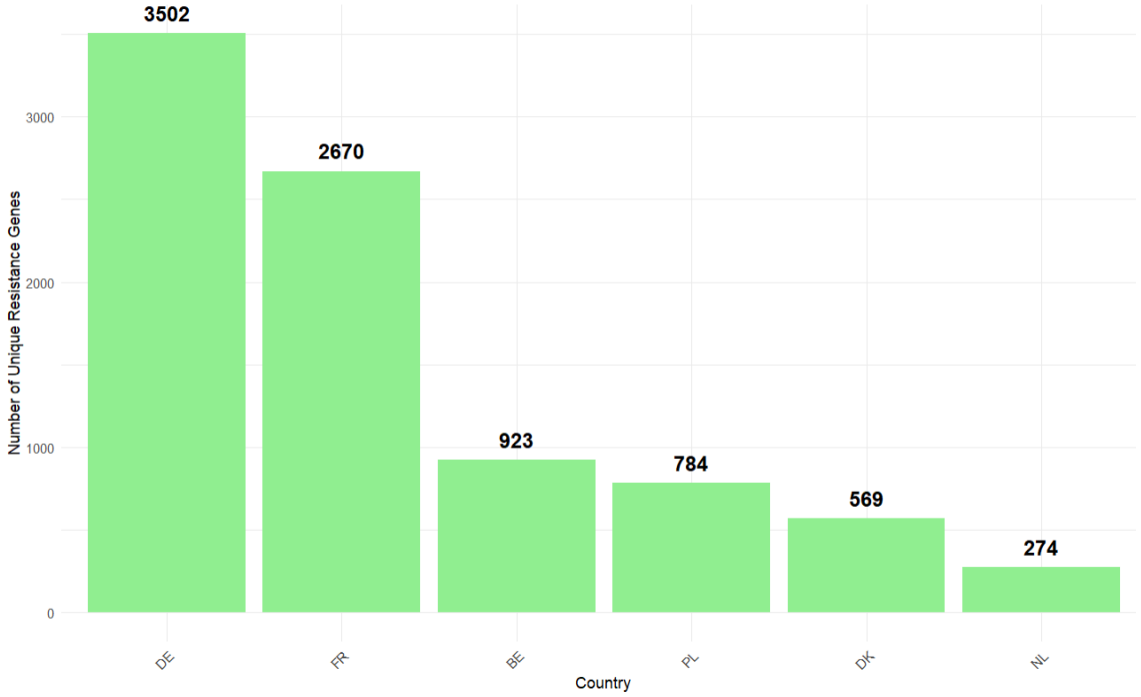

Figure 6. Total number of antimicrobial resistance gene clusters detected per country (>=5 genes).

5.2.2 Resistance genes variation across Types of Diet

Next, we explored gene distribution across dietary habits. Results should be cautiously interpreted due to the disproportional representation of types of diet in the dataset.

To examine differences in resistance gene diversity across dietary habits, the total number of distinct antimicrobial resistance genes detected in each group was calculated and presented in Figure 7. Individuals following a Red and Poultry Meat Consumption (RPMC) diet presented the highest diversity, with 7,372 resistance gene detections. This was followed by the NRMC and NMC groups, with 603 and 450 gene detections, respectively. The group without dietary information (NA) showed the lowest diversity (n = 305).

These values reflect the cumulative number of gene detections across individuals within each dietary group and do not indicate the number of exclusive genes of this groups. Due to the unequal number of individuals per group, these results are presented descriptively and should be interpreted with caution.

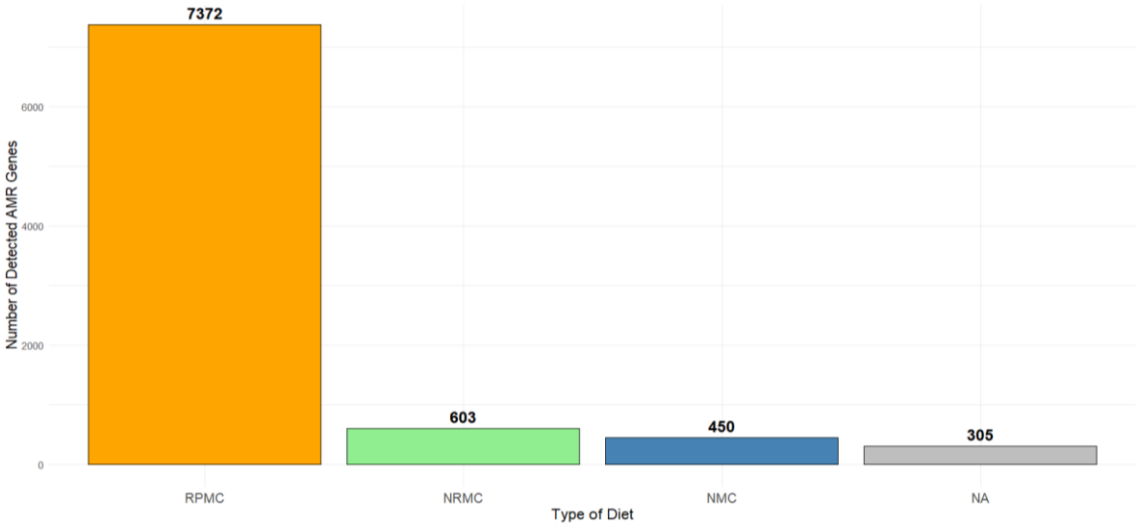

Figure 7. Total number of antimicrobial resistance gene clusters detected per type of diet.

5.2.3. AMR gene counts per Country and Type of Diet

The distribution of antimicrobial resistance (AMR) genes across countries and dietary groups revealed considerable variability in the number of resistance genes detected per group. Figure 8 represents the number of antimicrobial resistance genes that were identified per Country and Type of Diet showing that Germany presented the highest overall number of AMR genes (3,502), followed by France (2,670) and Belgium (923). In contrast, countries such as Denmark (569), the Netherlands (274), and others with lower sample sizes exhibited lower total counts of detected resistance genes.

Across all countries, individuals classified within the Red and Poultry Meat Consumption (RPMC) group accounted for most detected AMR genes. For instance, in Germany, 2,916 out of 3,502 genes were associated with RPMC individuals, while in France and Belgium, this group accounted for 2,501 and 923 genes, respectively. In Denmark and the Netherlands, all detections were likewise attributed to the RPMC group. Although these counts suggest a higher detection of AMR genes in RPMC individuals, it is important to note that this group also comprised the largest number of samples across countries, which may have contributed to the observed differences.

Resistance genes were also detected in other dietary groups. In Germany, 281 AMR genes were identified in individuals classified as No Meat Consumption (NMC), and 169 in France within the same group. NRMC individuals accounted for detections in the Netherlands and Poland (5 and 597 genes, respectively). These findings support the presence of resistance genes in individuals with diverse dietary profiles.

Overall, AMR gene detections occurred in all countries and across all dietary classifications. However, given the unequal number of samples per group and country,

the interpretation of these results remains descriptive. Further studies with balanced sampling and additional covariates would be necessary to explore the potential contribution of dietary patterns or geographical context to the human resistome.

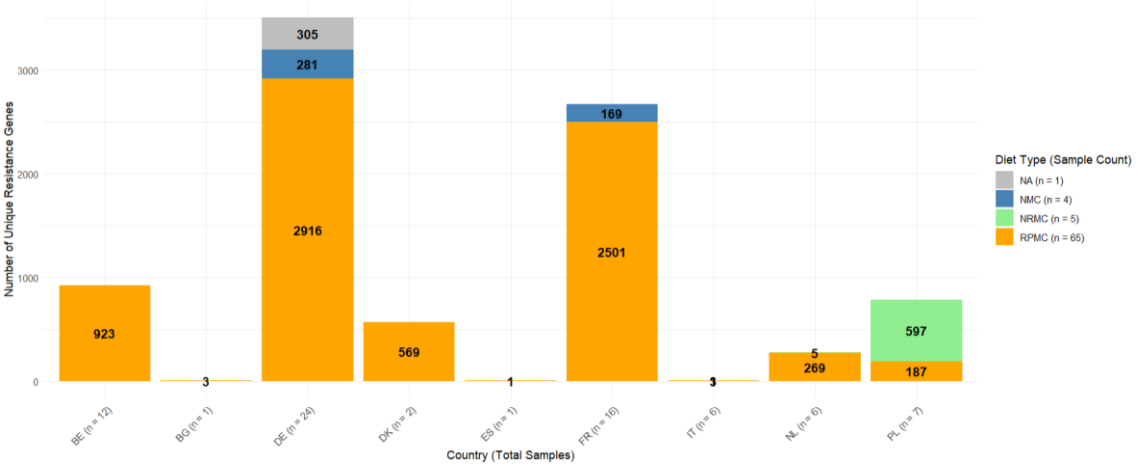

Figure 8. Number of antimicrobial resistance genes identified per Country and Type of Diet.

5.2.4. Distribution of antimicrobial resistance classes across countries and Types of Diet

Resistance genes associated with 19 antibiotic classes were identified across all analysed countries and dietary groups, reflecting their widespread presence in the human gut microbiome. The number and diversity of resistance classes were consistent across countries and diets, with no notable differences observed between groups.

When analysed by dietary habits, all groups, Red and Poultry Meat Consumption (RPMC), No Red Meat Consumption (NRMC), and No Meat Consumption (NMC), exhibited resistance genes belonging to all 19 antibiotic classes. Similarly, all countries included in the analysis showed the same class-level diversity, further supporting the broad dissemination of AMR genes regardless of geography or diet.

This uniformity was also observed when analysing country and diet jointly, with all combinations displaying resistance genes from the full range of antibiotic classes considered in this study. These results reinforce the notion that resistance gene diversity at the class level is widespread and not exclusively shaped by dietary or geographical factors.

5.3. Relative Abundance of Resistance Gene Clusters

While the previous sections focused on the detection and distribution of AMR genes based on their presence and count across countries and dietary groups, these measures do not consider the microbial context in which such genes occur. To address

this, we applied an additive log-ratio (ALR) transformation to assess the relative abundance of resistance gene clusters, normalizing gene counts in relation to bacterial composition within each sample.

This approach enables a more standardized comparison of AMR gene abundance across samples, accounting for differences in microbial load and sequencing depth. The following sections explore the distribution of AMR gene relative abundance across dietary patterns and geographical locations, aiming to identify compositional patterns in the human resistome beyond raw detection counts.

5.3.1. Bacterial Diversity Across Samples

To contextualize the abundance of antimicrobial resistance (AMR) genes, we performed taxonomic classification of the gut microbiome using Kraken, based on bacterial DNA sequences. Figure 9 illustrates an overview of the bacterial taxa detected across all samples, highlighting the complexity of the microbial community structure within the studied population.

Although this taxonomic profile offers a broad visual representation of microbial diversity, it was not directly used in the analysis of AMR gene abundance. The normalization applied through additive log-ratio (ALR) transformation was based on total bacterial counts, independent of taxonomic classification.

As such, Figure 9 is presented for illustrative purposes only, considering the full dataset, and no interpretations regarding differences in microbiome composition across countries or dietary groups were explored.

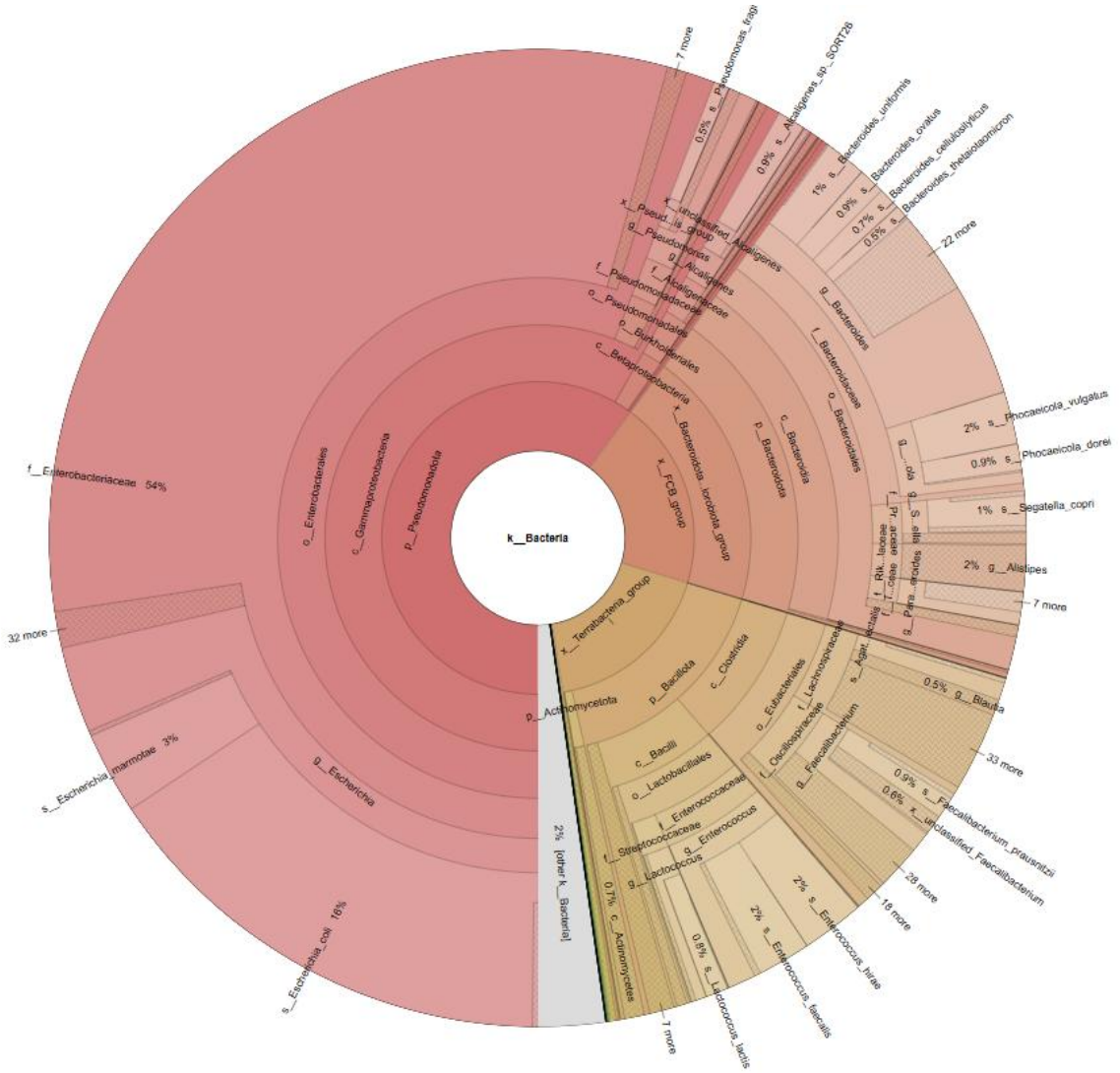

Figure 9. Taxonomic distribution of bacteria identified from human microbiome samples, classified with Kraken and visualized using a Krona chart. Each ring represents a different taxonomic level (from phylum down to genus/species), and the size of each sector indicates the relative abundance of that bacterial group.

5.3.2. AMR abundance across Types of Diet

As shown in Figure 10, the relative abundance of AMR genes varied across dietary groups, with the highest abundance observed in individuals following a Red and Poultry Meat Consumption (RPMC) diet (-10.8 ALR), followed closely by those adhering to a No Meat Consumption (NMC) diet (-10.9 ALR). No Red Meat Consumption (NRMC) individuals exhibited the lowest relative abundance (-11.2 ALR).

Less negative ALR values indicate higher relative abundance of resistance genes, while more negative values suggest lower abundance in proportion to bacterial counts. Despite these observed variations, the narrow range of ALR values across

dietary groups suggests that factors beyond diet are also likely influential in shaping observed resistance gene abundances. Although ALR-transformed AMR abundances varied slightly across diet groups, the narrow ALR range and small number of samples limit drawing definitive conclusions from these observations.

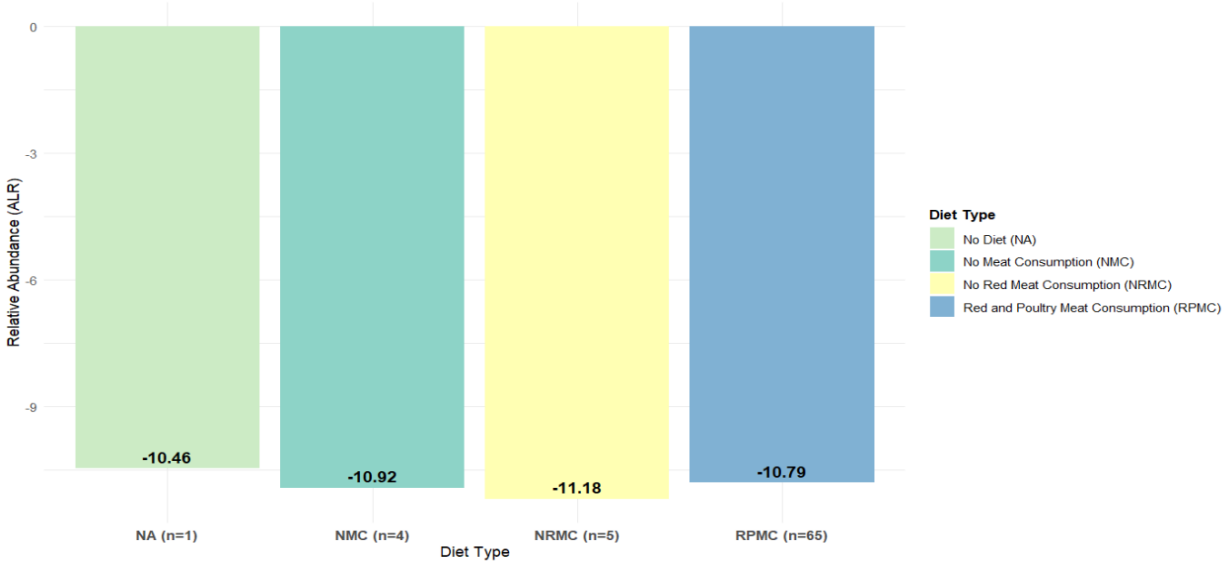

Figure 10. Relative abundance of antimicrobial resistance genes by dietary type

5.3.3. Country-wise variation in AMR abundance

Figure 11 shows the mean ALR values for each country, calculated from AMR-positive samples only. Among the countries analysed, Germany (-10.71), Denmark (-10.36), Belgium (-10.64), and the Netherlands (-10.42) exhibited relatively higher ALR values compared to countries such as France (-11.16), Poland (-11.49), Italy (-17.49), Bulgaria (-18.03), and Spain (-18.06). However, due to the limited and unequal number of AMR-positive samples per country (ranging from n = 1 to n = 24), these descriptive differences should be interpreted with caution.

These findings illustrate that, even after normalization for bacterial load, the relative abundance of AMR genes varies across countries. Yet, no statistical testing was applied, and further investigation with larger, balanced sample sizes would be necessary to assess whether such differences reflect broader geographical patterns or are influenced by sample-level variability.

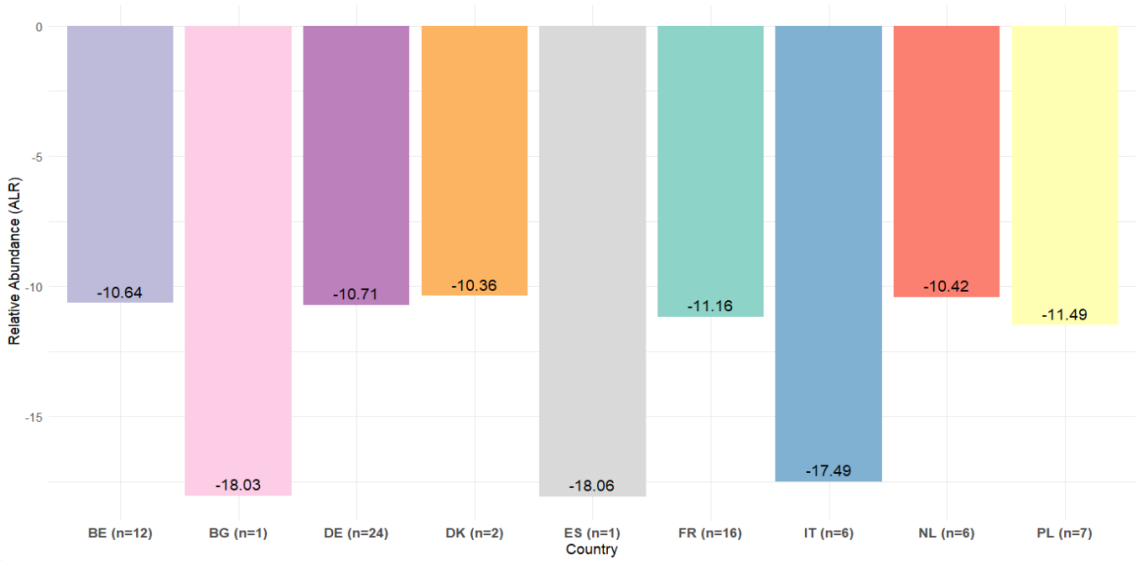

Figure 11. Relative abundance of antimicrobial resistance genes by country

5.3.4. Relative abundance across Country and Type of Diet

Figure 12 presents the relative abundance of antimicrobial resistance (AMR) genes across countries and dietary groups, based on ALR-transformed values. Each bar represents the average ALR for individuals classified as AMR-positive within a specific country and dietary category.

Across most countries, individuals classified under the Red and Poultry Meat Consumption (RPMC) group presented higher ALR values (i.e., less negative), compared to other diet types. In Germany and France, for example, the RPMC group exhibited a higher ALR than both the No Meat Consumption (NMC) group. The Netherlands showed the most distinct difference between dietary groups, with the RPMC group presenting a higher ALR compared to NRMC. Italy showed a similar, but less pronounced patterns. In Poland, in contrast, ALR values for NRMC were lower than those for RPMC.

Not all countries had representation across all dietary categories. For example, only RPMC individuals were present among AMR-positive samples in Belgium, Bulgaria, Denmark and Spain. The number of samples within each group varied substantially, with some combinations represented by only one or two individuals. This limits the interpretability of the differences and highlights the descriptive nature of these results.

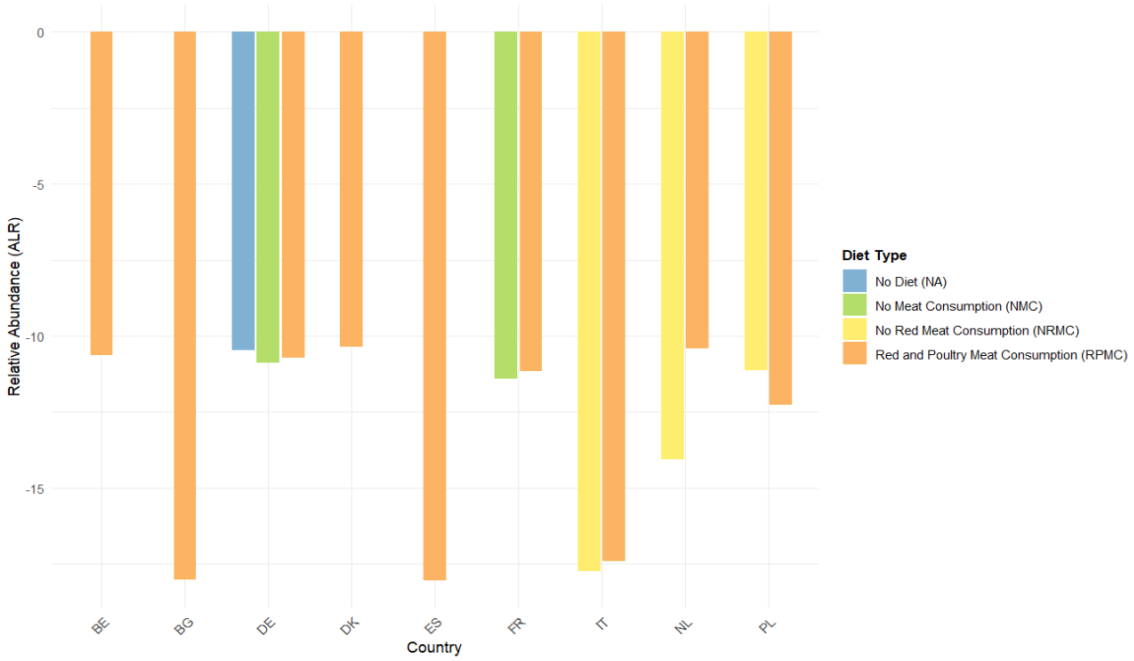

Figure 12. Relative abundance of antimicrobial resistance genes by country and dietary type.

5.4 Relative Abundance in the Antibiotic Classes

5.4.1. Relative Abundance per Antibiotic class

Lastly, the relative abundance of AMR genes by antibiotic classes revealed consistent distribution patterns across diets and countries; however, due to sample size limitations, these findings should remain strictly descriptive.

Figure 13 presents the ALR-transformed mean relative abundance of AMR gene clusters across all antibiotic classes detected in the dataset. The values represent the average proportional abundance of gene clusters associated with each class, normalized by bacterial load.

The class beta-Lactam exhibited the highest relative abundance, with an ALR of -12.3, followed by "Other" (-12.4) and Tetracyclines (-13.2). Several other classes also presented relatively high ALR values, including Quinolones (-13.4), Polymyxins (-13.7), and glycopeptides (-13.7).

Conversely, lower relative abundance values were observed for classes such as Pseudomonic acids (-17.3), Streptogramins (-16.6), Pleuromutilins (-16.9), and Phosphonic acid derivatives (-16.6). These classes showed the most negative ALR values among the group, indicating lower proportional abundance across the analysed samples.

Overall, ALR values ranged from -12.3 to -17.3 across the 19 antibiotic classes included in the analysis. These results describe the distribution of resistance gene

clusters in the studied human microbiomes at the antibiotic class level, based on relative abundance normalized to bacterial load and gene length.

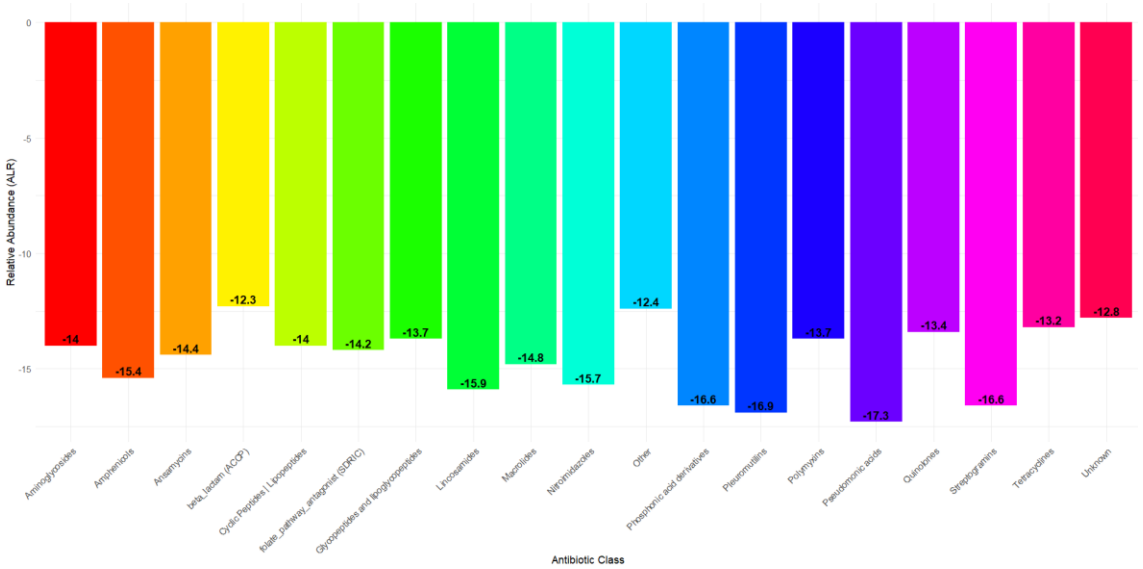

Figure 13. Relative abundance of AMR genes per antibiotic class (ALR-transformed).

5.4.2. Relative Abundance per Antibiotic Class per Type of Diet

Figure 14 presents the ALR-transformed relative abundance of antimicrobial resistance genes across five antibiotic classes, stratified by dietary group. Each bar represents the mean ALR value among AMR-positive individuals in each diet category.

Across all diet groups, beta-lactam (ACCP) resistance genes consistently showed the highest relative abundance, with ALR values ranging from -12.8 in the No Red Meat Consumption (NRMC) group to -12.3 in the Red and Poultry Meat Consumption (RPMC) group. Quinolone resistance genes have a slightly higher relative abundance observed in the NMC group compared to the other dietary categories.

Tetracycline resistance genes showed the second highest relative abundance in the RPMC group, with ALR values differing between meat consumers and non-consumers. The remaining classes, glycopeptides and lipoglycopeptides and polymyxins, showed relatively similar ALR values across all diet types, with variation remaining within a narrow range (approximately -13.6 to -14.3).

It is important to note that the number of AMR-positive resistomes per dietary category varied substantially, with the RPMC group comprising most samples. The NA group was represented by only one individual, and the NMC and NRMC groups included three and four individuals, respectively. As such, these results are presented descriptively and should be interpreted with caution.

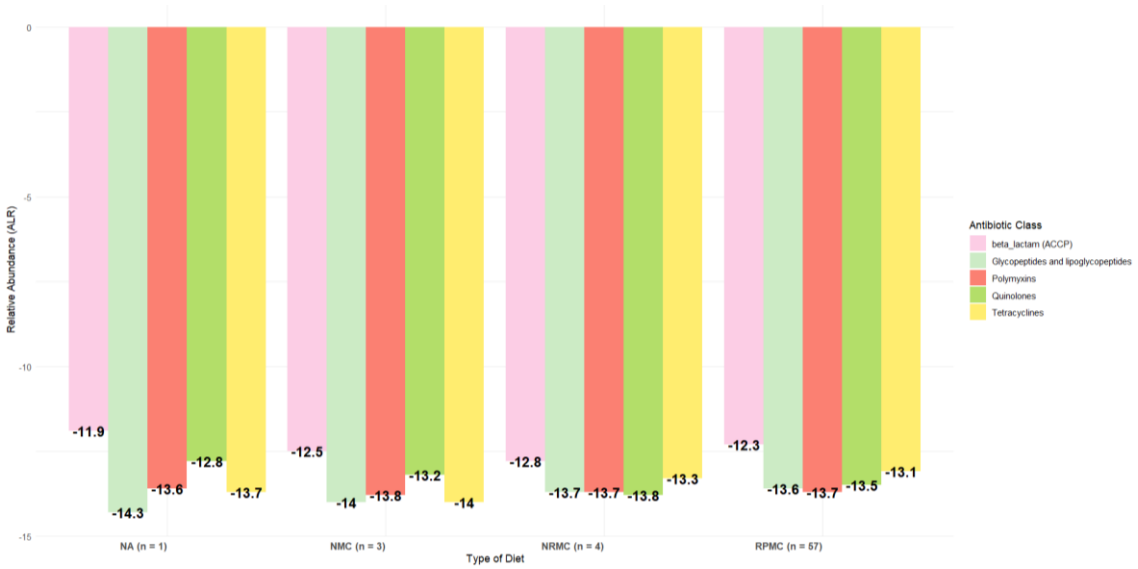

Figure 14. Relative abundance of AMR genes per antibiotic class (ALR-transformed) per Type of Diet

6. Discussion

This study aimed to characterize antimicrobial resistance (AMR) gene distribution across European human gut microbiomes, highlighting potential variations associated with dietary habits and geographical origin. Given the descriptive nature and sample size constraints, findings remain indicative, providing a comparative basis aligned with existing scientific literature.

The results indicate possible geographic and diet differences in AMR gene abundance. Participants with omnivorous diets (RPMC) seemed to carry relatively more AMR genes than those with vegetarian/vegan (NMC) or low meat diets (NRMC). Geographic variation in AMR gene relative abundance was observed. Countries such as Denmark, Germany, Belgium, and the Netherlands showed higher relative abundance when compared to Italy, Bulgaria and Spain that presented lower relative values.

Countries variations in the abundance of AMR may reflect country-level policies on antibiotic use, agricultural practices, and antibiotic stewardship in health care. According to the ESAC-Net report (ECDC, 2024), Germany, Belgium, and France have historically had more antibiotic use, particularly of beta-lactams and tetracyclines, compared to Denmark and the Netherlands, which have more restrictive policies of antibiotic use (ECDC, 2024).These patterns do not fully align with reported antibiotic use profiles. For example, both the Netherlands and Denmark maintain restrictive national antibiotic policies yet displayed relatively high AMR gene abundance in our dataset. However, the small number of AMR-positive samples and the uneven distribution of samples across countries limit the interpretability of these findings, as some of the observed differences may reflect sampling bias rather than true population-level variation.

Moreover, resistome composition is likely influenced by additional factors beyond national antibiotic consumption, including sample size and data structure (Gloor et al. 2017), individual antibiotic exposure (Aslam et al. 2021), environmental contact with resistant bacteria (Berendonk et al. 2015), dietary effects on the gut microbiome (David et al. 2014), and exposure to animal products or intensive production systems (Munk et al. 2018).

Our findings agree with existing evidence indicating likely dietary determinants of AMR exposure. Research indicates how meat-inclusive diets are associated with higher presence of AMR genes, that might be related to the presence of residual antibiotics and antibiotic-resistant bacteria commonly found in intensive livestock production systems, where antibiotics have historically been used for both prophylactic and therapeutic

purposes (Weinroth et al. 2022b; Fernández-Trapote et al. 2024). Analysis further revealed that the relative abundance of tetracycline resistance genes was higher among participants adhering to meat-inclusive diets (RPMC) versus vegetarian/vegan diets (NMC). The distinction is consistent with accounts of widespread tetracycline use in animal production, where the drug is extensively used for therapy and prophylaxis (Odey et al. 2024).The high occurrence of beta-lactam and tetracycline resistance genes may be mirrored to their extensive use in clinical medicine, veterinary medicine, and agriculture across Europe. European Food Safety Authority reports reveal that beta-lactams and tetracyclines are the most used ABs, with constant detection of their resistance genes in the environment and human microbiomes (ECDC 2022; EFSA 2024). The occurrence of these resistance determinants in human gut metagenomes illustrates the linked nature of human, animal, and environmental AMR reservoirs and strengthens the case for inclusive AMR surveillance under the One Health paradigm (van Schaik 2015; Munk et al. 2018). The correlation between diet and gut microbiome bacterial composition offers a reasonable explanation for differences in AMR abundance between diet groups (Wu et al. 2011).

Plant-based diets such as vegetarian or vegan have been previously associated with lower carriage of antimicrobial resistance (AMR) genes and greater microbial richness, potentially due to reduced exposure to antibiotic residues and higher intake of dietary fibre (David et al. 2014; Conlon and Bird 2015). In contrast, diets including red and poultry meat have been linked to increased AMR gene presence (Weinroth et al. 2022b). Although our study did not evaluate bacterial composition or diversity between dietary groups, the observed relative abundance of AMR genes was numerically lower in individuals following plant-based or restricted-meat diets. These findings may align with previously reported associations but given the limited number of AMR-positive samples in non-meat diet groups and the lack of taxonomic profiling in our analysis, these observations must be interpreted with caution.

Due to the low number of AMR-positive samples per dietary group, it was not possible to investigate associations between bacterial diversity and resistome composition. The percentage of AMR-positive samples in the dataset was limited, and as such, the current observations are best interpreted as exploratory. These descriptive patterns should be validated in future studies with larger and more balanced sample sizes. Additional functional metagenomic studies are necessary to ascertain if the detected resistance genes are being actively expressed and thus confer functional

relevance beyond their mere presence in the microbiome as part of the resistome (Berendonk et al. 2015)

Major limitations of this study include the low number of AMR-positive samples, which limits the power and generalizability of the results, as well as the opportunity to perform statistical testing. Uneven sampling between nations and diet groups offers prospective biases, compromising representativeness and possibly affecting observed patterns of AMR distribution. American Gut Project metadata lack information about antibiotic use, socioeconomic status, and access to healthcare, and these unmeasured factors could moderate or account for the reported AMR patterns. Not testing hypotheses statistically limits interpretative inferences, making our findings exploratory instead of confirmatory. Additionally, dietary metadata in open data can be coarse-grained, since self-reported diets do not capture heterogeneity in food sourcing, processing, or cooking, which can also influence AMR gene acquisition. With these limitations in mind, all interpretations must be considered preliminary, as a basis for informing future, more granular research. Follow-up studies should involve larger, more balanced datasets to enhance statistical power and generalizability. Longitudinal studies incorporating detailed environmental, dietary, and medical histories could provide a more integrated view of the complex interactions affecting the human resistome.

Nevertheless, the observed possible connection between diet and the abundance of AMR in the human gut microbiome suggests pressing opportunities for food and public health policy measures.

Interdisciplinary human, animal, and environmental One Health AMR surveillance remains central to study and control the transmission of resistance along food chains (Queenan et al. 2016; Munk et al. 2018). Recent efforts further illustrate the utility of metagenomic frameworks to uncover such links (Fernández-Trapote et al. 2024).

7. Conclusion

The objective of this research was to describe the pattern of antimicrobial resistance (AMR) genes in European human gut microbiota and determine potential differences related to diet and geographical location. Despite some observed patterns, these findings are constrained by the limited sample size, uneven sampling among dietary groups and nations, and lack of statistical analysis, restricting the possibilities for inferential conclusions. Utilization of publicly available metagenomic data sets can also introduce uncontrollable biases, with influence on resistome composition.

Nevertheless, these findings indicate diet as a possible determinant of AMR transmission and highlight the necessity for more extensive AMR surveillance, possibly considering eating habits. Comprehensive AMR surveillance can be addressed only by an extensive, multidisciplinary strategy under the One Health paradigm, combining human, animal, and environmental data and taking into account agricultural antibiotic stewardship and food safety regulation.

To build on these findings, longitudinal studies involving more equitable recruitment of participants and incorporating comprehensive dietary, medical, and environmental histories are necessary. In this way, greater insight into the multifaceted interrelationships between the gut microbiome diversity and resistance profiles will be gained to inform evidence-based interventions to mitigate AMR.

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