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Impact of macrolide antibiotics on gut microbiota diversity with age-specific implications and scientific insights

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ABSTRACT

This review investigates the effects of macrolides on the gut microbiota across different age groups. Macrolides, widely used to treat various infections, have been shown to disrupt the gut microbiome, leading to reduced bacterial diversity and increased risks of antibiotic resistance. The review examines the general mechanisms of action by macrolides, highlighting their role in inhibiting bacterial protein synthesis and promoting antibiotic resistance through horizontal gene transfer and selective pressure. Additionally, the reviews also focus on transition of gut microbiota across different age groups. It also addresses the dysbiotic shift induced by macrolides and its recovery following antibiotic discontinuation. Factors contributing to macrolides resistance, including genetic mutations and environmental factors, are discussed. The focus has been on alternative therapeutic approaches highlighted to mitigate resistance. Overall, the review provides a comprehensive overview of the implications associated with macrolides on gut health and offers insights into managing and minimizing resistance development.

1. Introduction

The diverse population of microorganisms inhabiting the gastrointestinal tract is essential for preserving human health [1]. Numerous physiological functions, including digestion, immunological regulation, and the production of vital nutrients, depend on the action of these microbial community [2]. In addition, the gut microbiota plays a crucial role in the homeostasis of host immune response and also plays an important role in antibiotic resistance [3]. The gut microbiota orchestrates a comprehensive defense system through molecular interactions that regulate immune responses, reinforce epithelial integrity, and prevent pathogenic invasion. This in turn protects host against various health issues such as inflammatory disorders, metabolic diseases, and neuropsychiatric conditions [4].

The gut microbiota creates an environment that limits pathogenic colonization. It produces short-chain fatty acids (SCFAs), such as acetate, which lower gut pH and inhibit pathogen growth [5]. Surface proteins like pili and fimbriae on commensals such as Bacteroides and Firmicutes occupy epithelial adhesion sites, which prevents pathogenic adhesion [6]. Additionally, commensals produce bacteriocins like nisin and lacticin, which disrupt bacterial membranes and inhibit protein synthesis, thereby reducing harmful microbial populations [7].

Healthy gut is modulated by metabolites such as polysaccharide A (PSA) from *Bacteroides fragilis*, activating Toll-like receptor 2 (TLR2) on dendritic cells, inducing regulatory T cell differentiation and anti-inflammatory cytokine secretion [8,9]. SCFAs, including butyrate, regulate immune tolerance by inhibiting histone deacetylase, enhancing the expression of immune-regulatory genes, and balancing pro- and anti-inflammatory cytokines like IL-10, which mitigate inflammatory diseases, including IBD and rheumatoid arthritis [10]. Butyrate binds to G-protein-coupled receptors (GPCRs) such as GPR41 and GPR43, promoting tight junction protein expression, reducing intestinal

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permeability, and limiting systemic inflammation [11]. It also stimulates goblet cells to produce mucins, forming a protective mucus layer and activating AMP-activated protein kinase (AMPK), strengthening tight junctions and decreasing intestinal permeability [12–14]. Additionally, SCFAs influence metabolic pathways and gut-brain signalling, further contributing to overall health [15]. Thus, gut microbiota initiates nutrient competition, adhesion inhibition, immune modulation, and antimicrobial peptide production to prevent pathogenic colonization.

1.1. Impact of dysbiosis on immune function and metabolic health

Dysbiosis in the gut microbiota has been associated with series of health implications [16]. For instance, In cardiovascular health, gut microbiota secretes metabolites like trimethylamine-N-oxide (TMAO), SCFAs, and secondary bile acids which play significant roles in regulating various cardiovascular diseases. The elevated TMAO levels, derived from microbial metabolism, promote endothelial dysfunction and atherosclerosis, while reduced SCFA production contributes to hypertension and heart failure through inflammation and microbial translocation [17].

Similarly, Neurological disorders are closely linked to alterations in gut microbial composition. In case of parkinson's and alzheimer's diseases, there is a reduction in SCFA-producing bacteria and increased gut permeability exacerbate neuroinflammation and protein aggregation, contributing to the progression of these neurodegenerative disorders [18]. Imbalance in microbiota affects the production of neurotransmitter precursors like tryptophan, which is converted into serotonin, there by influencing neuroinflammation and signalling via serotonin receptors [19,20]. Autism spectrum disorder is associated with disruptions in the gut microbiome that affect neurotransmitter production, while conditions such as multiple sclerosis and epilepsy are influenced by altered microbial profiles that modulate immune responses [21,22].

Where as, in metabolic disorders, microbial imbalances disrupt energy homeostasis, glucose metabolism, and lipid storage. These changes contribute to type 2 diabetes by promoting inflammation and insulin resistance, while compromised gut barrier integrity drives fatty liver disease [23,24]. Shifts in the ratio of Firmicutes to Bacteroidetes are associated with obesity, and systemic metabolic disruptions contribute to metabolic syndrome [25]. Dysregulated bile acid metabolism results in the conversion of primary bile acids into secondary bile acids such as, deoxycholic acid (DCA) and lithocholic acid (LCA) that disrupts glucose and lipid metabolism by affecting receptors like farnesoid X receptor (FXR) and TGR5, leading to insulin resistance and non-alcoholic fatty liver disease [26,27].

In case of autoimmune diseases further emphasize is attributed to role of gut microbes in immune modulation. Altered microbial communities in rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis elevated inflammation and disease severity [28]. Similarly, immune dysregulation in lupus and celiac disease has been linked to gut microbiota changes [29].

Emerging evidence highlights the microbial contribution to cancer, chronic kidney disease, and respiratory illnesses [30,31]. Reduced SCFA-producing bacteria and increased uremic toxin producers, such as indoxyl sulfate, p-cresyl sulfate, and TMAO, compromise the gut barrier. This facilitates toxin translocation into the bloodstream, contributing to systemic inflammation and kidney dysfunction [31]. The gut-lung axis connects microbial alterations to asthma and chronic obstructive pulmonary disease through inflammatory pathways [32].

Apart from these above-mentioned health implications, the expansion of drug resistant-pathogens has significantly influenced the gut diversity. This can be attributed to in appropriate usage of different antibiotics [3]. One such classic example includes increasing prevalence of macrolide administration among different ages [33].

1.2. Macrolides and its clinical applications

Macrolide antibiotics are classified as antibiotics drugs with characteristic of macrocyclic lactone ring structures [34]. The usage of macrolides was expanded with advanced scientific knowledge which led to the discovery of erythromycin and its semisynthetic derivatives, including azithromycin, roxithromycin, clarithromycin, and telithromycin. These drugs are widely used due to their broad-spectrum activity against a range of bacterial infections [35]. Clinically, macrolides are employed to treat respiratory tract infections, skin infections, and sexually transmitted infections, and are also used prophylactically at targeted-risk populations [36].

Erythromycin, a broad-spectrum macrolide, is effective against a variety of bacterial infections, including skin infections, streptococcal pharyngitis, and pneumonia caused by *Legionella* and *Chlamydia*. It is also beneficial for treating gastrointestinal dysmotility and is used prophylactically in orthopedic surgery [37–39]. Advancements in nanoparticle formulations aim to enhance its bioavailability and solubility [40].

Azithromycin, another macrolide, is commonly used for respiratory infections, including pneumonia, acute bronchitis, and otitis media. It is also effective against acute intestinal infections and Lyme borreliosis [41,42]. Azithromycin inhibits bacterial protein synthesis and enhances immune responses, though its misuse has led to increasing bacterial resistance [43].

Clarithromycin is widely used for respiratory infections caused by *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella* species. It is effective in treating upper and lower respiratory infections [44,45]. It has also shown efficacy in reducing sepsis recurrence and restoring immune function [46].

Dirithromycin is used for respiratory and skin infections, including chronic rhinitis, asthma, and chronic obstructive pulmonary disease. It has shown high efficacy in treating bacterial exacerbations of chronic bronchitis and skin infections caused by *Staphylococcus aureus* and *Streptococcus pneumoniae* [47–49].

Roxithromycin is effective in managing chronic sinusitis, dental infections, allergic rhinitis, and respiratory infections. It improves symptoms of chronic sinusitis, reduces nasal polyps, and is highly effective in dental infections [50,51]. Roxithromycin showed 89 % success in treating lower respiratory infections, comparable to erythromycin and doxycycline [52], and achieved an 89 % cure rate in pediatric cases [53].

Recently introduced and merging macrolides, such as telithromycin, cethromycin, and solithromycin, are reported to be effective against respiratory pathogens, particularly resistant *Streptococcus pneumoniae* strains. Telithromycin is approved for community-acquired pneumonia and bronchitis, while cethromycin and solithromycin are under investigation [54,55]. These macrolides highlight the adaptability of this antibiotic class in treating diverse bacterial infections across clinical settings.

Despite their benefits, they are linked to both immediate and long-term adverse health effects, such as increased risks of necrotizing enterocolitis, bronchial hypersensitivity, asthma, obesity, and autoimmune diseases [56]. Macrolides are also reported to disrupt which eventually imbalance the microbial diversity. The disruption can favour opportunistic pathogens to colonize which can lead to the development of antibiotic resistance [57].

The interaction between macrolides and the gut microbiota is highly critical as they target both normal flora and pathogens [58]. This action can impair vital physiological processes such as digestion, nutrient absorption, and immune system function, highlighting the complex relationship between antibiotic use and gut health [59]. Furthermore, the perturbation of the gut microbiota by macrolides may facilitate the emergence and proliferation of multi-drug resistant pathogens which pose significant challenges to both individual and public health [58]. Thus, understanding the effects of macrolide antibiotics on the gut microbiota is essential for developing strategies to minimize adverse

effect and improve health condition.

The aim of this study is to investigate the age-specific effects of macrolides on gut microbiota in infants, children, adults, and the elderly. The study will explore how macrolides contribute to the development of antibiotic resistance, focusing on their impact on microbial diversity and the mechanisms underlying resistance. It will also assess the dysbiotic shifts induced by macrolides and the recovery of gut microbiota after antibiotic discontinuation. Additionally, the study aims to evaluate strategies to mitigate the negative effects of macrolides on gut health and reduce the risk of resistance across different age groups.

2. General mechanisms of action by macrolides

Macrolides are well-known for their ability to inhibit bacterial protein synthesis, which is essential for bacterial growth and replication. This inhibition prevents bacteria from producing proteins necessary for their survival, effectively leading to their death [35]. Macrolides inhibit bacterial protein synthesis through a specific and detailed mechanism involving the bacterial ribosome. Macrolides specifically target the 50S subunit, where they bind to the 23S rRNA [60]. This binding occurs within the peptidyl transferase center (PTC) of the ribosome, a critical site for peptide bond formation during protein elongation [61]. When macrolides bind to the 23S rRNA in the PTC, they obstruct the polypeptide exit tunnel [62]. This tunnel is the pathway through which newly synthesized polypeptide chains exit the ribosome. The obstruction caused by macrolides prevents the elongating polypeptide from passing through this tunnel, thereby halting the elongation process. Consequently, the nascent polypeptide chain cannot be completed, and protein synthesis is effectively terminated [62].

The blockade of the polypeptide exit tunnel by macrolides has several downstream effects on bacterial cells. It leads to the accumulation of incomplete polypeptides within the ribosome, which can interfere with ribosomal function and further inhibit protein synthesis [63]. This leads to the inability to produce essential proteins disrupts various cellular processes, including metabolism, cell wall synthesis, and DNA replication, which are critical for bacterial growth and survival. Without these vital proteins, bacterial cells cannot maintain their structural integrity or perform necessary metabolic functions, leading to cell death [63].

Additionally, the binding of macrolides to the ribosome can induce a conformational change in the ribosomal RNA and proteins, further impairing the ribosome's ability to synthesize proteins. This conformational change can enhance the binding affinity of macrolides, making them more effective at lower concentrations [63,64]. The specificity of macrolides for the bacterial ribosome, as opposed to the eukaryotic ribosome, accounts for their selective toxicity towards bacteria, making them highly effective antibacterial agents [65]. The general mechanism of action by macrolides is shown in Fig. 1.

3. Development of antibiotic resistance to macrolides

A primary mechanism by which bacteria develop resistance to macrolides involves alterations to the antibiotic's binding site on the ribosome. This alteration is often due to the methylation of adenine residues in the 23S rRNA of the 50S ribosomal subunit, the macrolide binding site [66]. This process is mediated by rRNA methylase enzymes encoded by the erythromycin ribosome methylation genes -erm genes. Methylation of the binding site diminishes the binding affinity of macrolides, preventing them from effectively inhibiting protein synthesis and thereby conferring resistance [66].

The efflux pumps are another significant mechanism of resistance. These transmembrane proteins actively transport macrolide molecules out of the bacterial cell, thereby reducing the intracellular concentration of the antibiotic [66]. This mechanism is often mediated by genes such as Mef (macrolide efflux) and Msr, which encode specific efflux pumps. The Mef genes encode specific transporters within the major facilitator superfamily MSF and Msr encodes for an ATP binding cassette protein that contains two fused nucleotide-binding domains without any membrane-spanning regions [67]. For example, the MefA-MsrD gene in *Streptococcus pneumoniae* encodes an efflux pump that specifically expels macrolides, conferring resistance [68]. Further, In *Escherichia coli*, a trans-dominant negative mutation in the ABC protein Msr(D) affects the MFS transporter Mef(E), suggesting that these proteins can interact in vivo. This represents a functional interaction between an ABC component Msr and MSF transporter in the efflux of macrolides [67]. This resistance mechanism is commonly recognized by the observation that the amount of antibiotic accumulated inside resistant bacterial cells increases to levels comparable to those in susceptible cells in the presence of uncoupling agents such as arsenate, 2,4-dinitrophenol (DNP), and carbonylcyanide-m-chlorophenylhydrazone (CCCP). This indicates that the efflux pumps, which rely on energy either from ATP or a proton-motive force, are critical in mediating resistance [66].

Resistance develops due to the enzymes produced by bacteria that chemically inactivate macrolide antibiotics. These enzymes modify the macrolide molecules, rendering them ineffective. Esterases, encoded by the Ere gene, hydrolyze the lactone ring of macrolides, while phosphotransferases, encoded by the Mph gene, add phosphate groups to the antibiotic [66]. The **erm** gene encodes a methyltransferase enzyme, that methylates the adenine residue in the 23S rRNA of the 50S ribosomal subunit [69]. For instance, a genomic epidemiology study on *Salmonella* and *E. coli* identified the presence of resistance genes such as Mph(A), Mph(B), Mef(B), erm(B), and Mef(C)-Mph(G). Other resistance genes

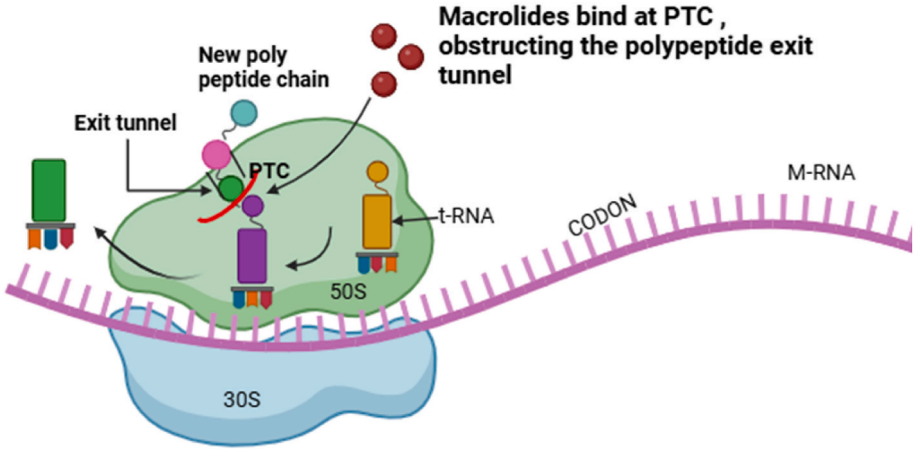

Fig. 1. Mechanism of macrolide action in inhibiting protein synthesis.

found in *E. Coli* include mph(E)-Msr(E), erm(C), erm(42), and ere(A). In 69 % of these isolates, the presence of these macrolide resistance genes either singly or in different combinations correlated with the azithromycin-resistant phenotype [70]. Further, the clinical efficacy of macrolides is reported to compromise by resistance mechanisms such as mutations in ribosomal at the drug binding site and alterations in ribosomal protein. The erythromycin efficacy is compromised by mutations in ribosomal RNA and alterations in ribosomal protein uL22. Cryo-EM studies of *Staphylococcus aureus* 70S ribosomes with a uL22 β hairpin loop deletion mutation reveal that these changes create a wider protein exit tunnel, allowing nascent proteins to bypass the erythromycin blockade, thus conferring drug resistance [71]. These resistance mechanisms collectively contribute to the increasing prevalence of macrolide-resistant bacterial strains as shown in Fig. 2.

4. Macrolides and the dysbiotic shift in gut microbiota

Macrolides disrupt microbial communities in the gut microflora by depleting microorganism-associated molecular patterns (MAMPs), impairing pattern recognition receptor (PRR) pathways such as TLRs and NLRs. This weakens key signaling cascades like NF-κB activation, reducing proinflammatory cytokines such as TNF-α and compromising mucosal immunity and homeostasis [72].

In animal studies also demonstrated the influence of macrolides on their gut. macrolides negatively impact lymphoid tissue development, T cell differentiation, and antimicrobial peptide (AMP) production. Specifically, they target microbial taxa crucial for T cell activation, notably affecting Th17 cell development and reducing the expression of IL-17 and β-defensins [73]. The loss of beneficial bacteria, such as segmented filamentous bacteria (SFB), further increases susceptibility to pathogenic invasion and intestinal infections [73].

Macrolides are also known to alter immune gene expression, particularly affecting the bactericidal lectin Reg3γ, which depends on LPS signals from commensals in Paneth and intestinal epithelial cells [74]. Additionally, macrolide treatment diminishes neutrophil recruitment and IL-17 production, heightening susceptibility to infections like sepsis caused by *Escherichia coli* and *Klebsiella pneumoniae* [75].

On a systemic level, macrolides influence T lymphocyte activity by reducing $CD4^+$ and $CD8^+$ T cell populations and lowering cytokine levels, including IFN-γ, TNF-α, and IL-17, which weakens immune responses [76,77]. These effects are partly mediated through alterations in the gut microbiota, which play a critical role in regulating immune homeostasis. A decline in immunoglobulin levels, particularly IgG, further highlights the negative impact on systemic and gut-associated immunity [77]. Additionally, macrolide treatment activates dendritic cell migration and the Th2-IL-4-IgE signalling pathway, potentially promoting inflammation and allergic responses, linked to gut microbial alteration [78]. These findings emphasize the significant impact of macrolides on gut microbiota-regulated immune functions, leading to local and systemic immune impairments and increased vulnerability to infections. The alteration of gut microbial composition and immune signalling pathways illustrates the extensive consequences of macrolide exposure.

5. The transition of gut microbiota across different age groups

5.1. Gut microbiota in infants and children

The development of the gut microbiota in infants and children is a dynamic process that begins at gestation age, birth, and continues to evolve through early childhood [79]. The idea of a sterile womb may be called into question by new research indicating that microbial sites can affect embryonic immune development prior to delivery [80]. The composition of the mother's gut microbiota prior to delivery is recognized to have a major effect on the immunity of the newborn [80]. The fetus's gut microbiota before birth is influenced by several maternal factors, including the mother's health and nutrition before and during pregnancy, pregnancy complications such as gestational diabetes, and the use of antibiotics by the mother [81]. After birth, the infant gut is colonized by bacteria from the mother and the environment. Factors affecting the progression of the infant microbiome, include delivery method, gestational age at birth, various antibiotic exposure during the perinatal period, and feeding practices such as breastfeeding versus formula feeding [82]. Factors affecting gut microbiota infants is shown is Fig. 3.

This early microbiota is relatively simple and is primarily composed

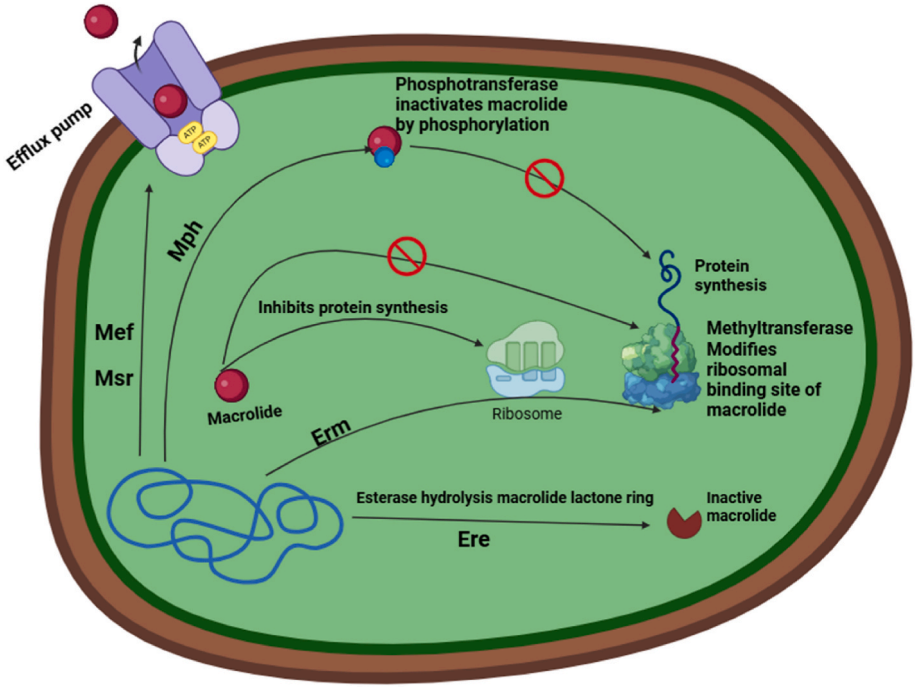

Fig. 2. Resistance genes causing macrolide resistance.

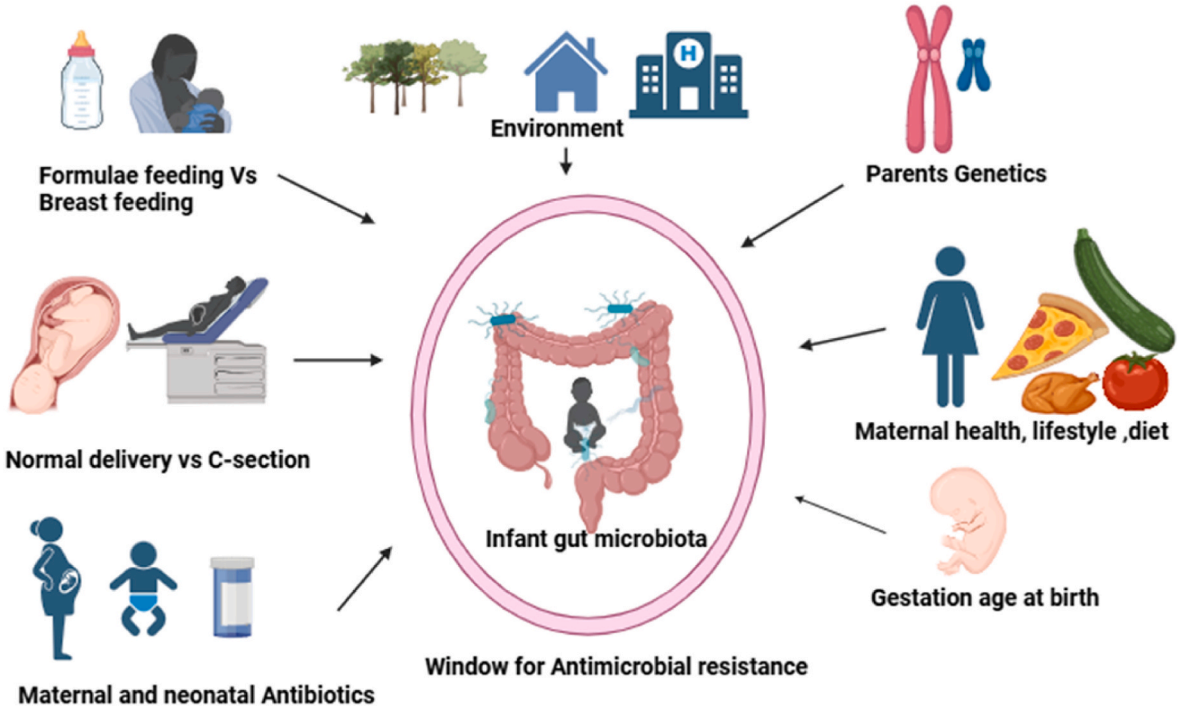

Fig. 3. Factors influencing neonatal microbiota and development of antimicrobial resistance.

of facultative anaerobes [83]. As the infant grows, the gut microbiota diversifies, with an increase in obligate anaerobes like Firmicutes, Bacteroides, Actinobacteria, and Proteobacteria [84]. Initially dominated by aerotolerant and facultative anaerobes like *Streptococcus*, *Enterococcus*, and *Lactobacillus*, the neonatal gut quickly shifts to anaerobic Bifidobacteriaceae in breastfed infants [85]. Throughout infancy and early childhood, other anaerobic families such as Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae become more abundant, shaping the taxonomy of the gut microbiota [85]. In infancy, exposure to new microbes increases diversity within the gut (alpha diversity). Initially unstable and sensitive to disruption compared to adults, the gut microbiota stabilizes and matures by around 3–5 years of age, resembling the stable microbiota found in adults. By this age, a child's gut microbiota resembles that of an adult, both in composition and functional capabilities [86].

5.2. Gut microbiota in adults

In adults, the human gut microbiome is stable over time and highly individualized in both its composition and functions, essential for maintaining overall health [87]. The adult gut microbiota is predominantly composed of bacteria from the phyla Firmicutes and Bacteroidetes, with Actinobacteria, Proteobacteria, and Verrucomicrobia [88]. This mature microbiota performs vital functions such as aiding in digestion, synthesizing vitamins, and modulating the immune system [88]. Unlike the rapidly developing and fluctuating gut microbiota in infants, the adult microbiota has reached a state of stability through years of diet, lifestyle, and environmental exposures [89]. The distal gut microbiota of teenagers between the ages of 11 and 18 was examined in a comparative observational study, and non-randomised samples from healthy adults were also compared. Based on the relative number of species, principal components analysis clearly distinguished between the adolescent and adult groups. All samples were dominated by *Clostridia* sp. A core microbiome consisting of 46 species was found in each sample, with taxa such as *Ruminococcus, Faecalibacterium*, and *Roseburia* standing out. Despite comparable rates of species detection, adolescents showed noticeably larger abundances of the genera Bifidobacterium and Clostridium than adults. These findings challenge the notion that adolescent and adult gut microbiomes are similar [90]. Defining site-specific baseline microbiomes linked to human gut, especially at lower taxonomic levels like genus, species, or strain, poses challenges [91].Primarily 16S rRNA gene amplicon sequencing, offer limited resolution. Structural differences resolved at higher taxonomic ranks (phylum, class, order) may not accurately reflect health correlations due to regrouping of diverse taxa [91].

5.3. Gut microbiota in elderly

The gut microbiome undergoes notable modifications in both composition and function as people age from adulthood to their elderly years. The microbiota's composition doesn't abruptly alter at a certain age rather, these changes occur gradually over time [92]. In adults, the gut microbiota typically maintains stability with a diverse and balanced community of bacteria crucial for digestion, immune function, and overall health, but when compared in the elderly, this stability diminishes, leading to significant alterations in the gut microbiota [88, 93]. Studies have shown that the elderly exhibit a reduction in microbial diversity and shifts in the relative abundance of key bacterial taxa [94]. Beneficial bacteria such as *Bifidobacterium* and Firmicutes often decrease, while potentially harmful bacteria belonging to Proteobacteria and certain *Clostridia* species increase [95]. This shift can be attributed to various factors, including changes in diet, reduced gut motility, altered immune function, and increased medication use.

The elderly population is more susceptible to dysbiosis, a state of microbial imbalance that can negatively impact health [94]. Age-related physiological changes, such as decreased stomach acid production, slower intestinal transit times, and weakened immune responses, contribute to this vulnerability [96]. Antibiotics, proton pump inhibitors, and nonsteroidal anti-inflammatory medicines are among the treatments that might further disrupt the gut microbiota when taken often. An array of detrimental health consequences, such as elevated inflammation, compromised nutritional absorption, and an elevated susceptibility to infections, have been linked to dysbiosis in the elderly [97]. The decline in beneficial bacteria that help maintain gut barrier

integrity can lead to conditions such as leaky gut syndrome, worsening systemic inflammation and potentially contributing to chronic diseases like cardiovascular disease, diabetes, and neurodegenerative disorders [94]. The factors influencing elderly gut microbiota are shown in Fig. 4. This imbalance can have far-reaching health implications, emphasizing the importance of monitoring and supporting gut health in the aging population. Strategies such as dietary interventions, prebiotics, probiotics, and careful medication management can help mitigate the negative effects of these microbial changes and promote a healthier gut microbiota in the elderly [98].

6. Influence of macrolides on gut microbiota in different age groups

Macrolides, generally known as bacteriostatic protein synthesis inhibitors, inhibited nearly all tested commensals and killed several species. The killed bacteria were more easily eliminated from in vitro communities than those merely inhibited. These species-specific killing challenges the traditional distinction between bactericidal and bacteriostatic antibiotics and may explain the significant impact of macrolides on gut microbiomes in both animals and humans [99]. Macrolides have impacts on the gut microbiota that go beyond their direct antibacterial activity. These antibiotics have the potential to significantly change the variety and constitution of the gut microbial ecosystem. The protein synthesis inhibition action of macrolides is intended to target pathogenic bacteria, but it also affects commensal bacteria residing in the gut [100]. The broad-spectrum nature of macrolides affects both harmful and beneficial bacteria, resulting in a significant impact on the overall microbial community within the gastrointestinal tract [101]. By disrupting the protein synthesis machinery in these beneficial bacteria, macrolides inadvertently contribute to the depletion of microbial

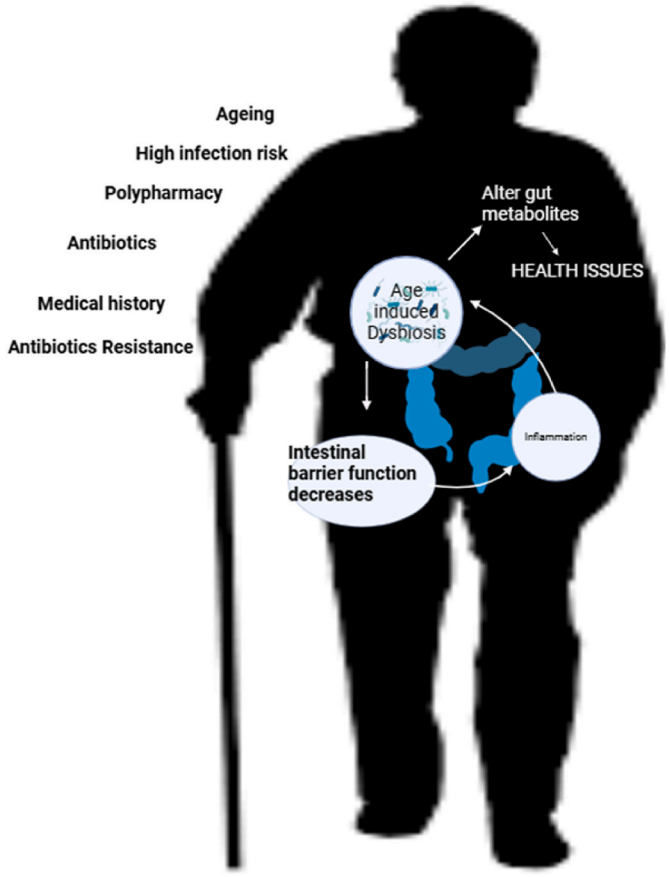

Fig. 4. Factors influencing gut microbiota in the elderly.

populations that are essential for maintaining a balanced and healthy gut microbiome. The impact on microbial diversity and composition is particularly concerning [102]. The effects of macrolides on gut microbiota vary by age group, with infants experiencing significant alterations in microbial diversity, children showing disruptions that may impact immune development, and adults exhibiting shifts that can lead to serious health implications. The adult gut microbiota's stability and ability to rebound highlight the critical differences in how antibiotics impact microbial communities at different life stages. Understanding these differences is essential for developing age-specific guidelines for antibiotic use to minimize adverse effects on the gut microbiota.

6.1. Macrolide impact on gut microbiota in infants and children

The administration of macrolide antibiotics during these critical developmental stages can have significant short and long-term impacts on the gut microbiota. Macrolides, such as azithromycin, are commonly used to treat Ureaplasma infections in preterm infants [103]. The resistance is often seen in their stool microbiota within the first month. A cross-sectional observational study found that 91 % of azithromycin-resistant bacteria carried at least one of six macrolide resistance genes: erm(A), erm(B), erm(C), erm(F), mef(A/E), and msr (A). Among 280 azithromycin-resistant isolates, *Staphylococcus* species dominated at 75 %, followed by *Enterococcus* species at 15 %. The most common resistance genes identified were erm(C) (46 %) and msr(A) (40 %), with 18 % of isolates carrying multiple resistance genes. This attenuates the risk of macrolide resistance in preterm infants due to the increasing use of these antibiotics in this population [104]. Reduced microbial diversity and changes in the relative abundance of important bacterial species are some of the immediate consequences. Children from 8 days to 59 months old participated in a randomized, placebo-controlled trial to investigate the effects of a single oral azithromycin treatment on their resistome and gut microbiota. 450 kids participated in the study, with an equal number in the azithromycin and placebo groups. Azithromycin dramatically decreased gut bacterial diversity and elevated genetic markers of macrolide resistance by 243 times as compared to placebo two weeks into the treatment. But after six months, these alterations were not noticed, suggesting that the benefits were only transient. Other antibiotic classes did not exhibit any changes in resistance, indicating that an azithromycin course did not result in co-resistance [105]. Further macrolide treatment can alter the gut microbiota by reducing beneficial Bifidobacteria and increasing potentially inflammatory *Clostridia* and *Enterobacteria* in infants. This shift may contribute to heightened inflammatory responses in infants, disrupting the gut's metabolic and immune functions [106]. Additionally, a quantitative, retrospective observational study analysed 200 patient records from 2019 to 2021 to verify gastrointestinal side effects in patients treated with macrolides. Among these patients, 24.5 % experienced side effects, including diarrhea, constipation, bloating, vomiting, and abdominal pain [107]. The data suggest a notable prevalence of gastrointestinal issues during macrolides treatment to children.

The early exposure to macrolides can have lasting effects on the gut microbiota's composition and function. The early-life exposure to macrolides like tylosin is associated with an increased risk of developing chronic conditions and susceptibility to bacterial infections later in life. Mice treated with a 5-day course of tylosin during early life, exposure resulted in greater microbiota disruption and severe colitis when exposed to pathogens [108]. Transfer of the antibiotic-altered microbiota to germ-free mice also exacerbated colitis, indicating that the altered microbiota alone was sufficient to increase susceptibility to the infection. These findings emphasize the hypothesis of the lasting impact of early-life antibiotic exposure on the gut microbiota and subsequent vulnerability to enteric pathogens in adulthood [108]. A longitudinal observational study including Finnish children aged 2–7 years revealed that the use of macrolides causes long-lasting alterations in the gut microbiota, such as decreased activity of bile-salt hydrolase, greater

resistance to macrolides, and an increase in Bacteroidetes and Proteobacteria. Additionally, early use is associated with increased risks of weight gain related to antibiotic use and asthma, with impacted children having unique microbiome profiles [109].

Several studies have investigated the specific changes in microbial taxa following macrolide treatment in infants and children. One study found that macrolide exposure significantly impacts gut microbiota, reducing richness for longer than penicillin. Studies show decreases in beneficial bacteria like Bifidobacteria and *Lactobacillus* and increases in Proteobacteria, including *E. coli* [101]. Macrolide treatment led to a prolonged decrease in the abundance of commensal species, which are crucial for maintaining gut health and preventing pathogen colonization [110]. *Bifidobacterium* species are crucial commensals in the infant gut, generally less prone to antimicrobial resistance genes compared to other taxa. Lower levels of *Bifidobacterium* are linked to higher antibiotic resistance gene loads, highlighting its role in the early development of antibiotic resistance in the gut [111] Moreover, metagenomic analyses have revealed that macrolide exposure can increase the prevalence of antibiotic-resistant genes within the gut microbiota. This horizontal gene transfer can promote the spread of resistant strains, not only affecting the treated individual but also posing a broader public health risk [111]. A meta-analysis found macrolides, such as azithromycin, significantly lower gut microbiome diversity. These results highlight the need to consider microbiome disruption when prescribing macrolides to children [101].

A genomic surveillance study explored azithromycin's impact on antibiotic resistance in *Escherichia coli* and *Salmonella* using whole genome sequencing (WGS) from isolates gathered in antimicrobial resistance (AMR) surveillance programs. The study involved 1007 *E. coli* isolates (165 resistant) and 269 *Salmonella* isolates (29 resistant), revealing key resistance genes such as mef(C)-mph(G). Additionally, the study found that azithromycin use in children promotes gut dysbiosis, leading to an increased risk of opportunistic infections. Metabolic profiling showed significant disruptions in key functions: decreases in protein modification, cofactors, prosthetic groups, electron carriers, vitamin biosynthesis, nucleic acid processing, secondary metabolite biosynthesis, and aldehyde degradation, while carbohydrate degradation and pentose phosphate pathway functions were enhanced. These findings emphasize the broader impact of macrolide treatment on children's gut microbiota and metabolic functions, alongside its role in driving antibiotic resistance [112].

Similarly, a cohort study in Bangladesh investigated the effects of oral antibiotic use, including macrolides, on the gut microbiome and antimicrobial resistance in 1093 infants. The results revealed that recent macrolide use within the past 7 days reduces microbiome diversity and increases the abundance of *Enterococcus* and *Escherichia/Shigella* species. This transient reconfiguration of the gut microbiome highlights macrolides' potential to influence antimicrobial resistance gene patterns in children [113]. These studies collectively underline the significant effects of macrolides on gut health, microbial composition, and resistance development in pediatric populations.

The high dissemination of macrolide-resistance genes in newborns demonstrates the rapid acquisition and spread of antibiotic resistance determinants early in life, mirroring the resistance patterns observed in paediatric populations where frequent exposure to macrolides contributes to the emergence and proliferation of resistant strains [114]. The widespread dissemination of macrolide resistance genes among newborns, detecting mef(A) in all infants, erm(B) in 58 %, and erm(C) in 50 %. It investigated early microbiome changes across different body sites within 72 h after birth. The research attenuates acquisition of antibiotic resistance genes (ARGs) during this short period, specifically observing prevalent macrolide resistance determinants and mec(A) expression across the sampled body sites [114].

Young children exposed to macrolides early in life may exhibit altered patterns of resistance that persist into later years [111]. A community-randomized study conducted across 30 Nigerian communities, azithromycin was widely given to preschoolers as part of the MORDOR I experiment, which was carried out in Tanzania, Malawi, and Niger. This resulted in a decrease in childhood mortality. However, one analysis of the gut microbiome of Nigerian children showed that treatment with azithromycin significantly decreased the number of pathogens, such as *Campylobacter* species, in the gut. Additionally, the gut microbiome of children receiving azithromycin showed elevated expression of the gene responsible for macrolide resistance, according to metagenomic and resistome analysis. These findings suggest that while azithromycin reduces childhood mortality, its prolonged use may promote the development of antibiotic resistance in gut bacteria, posing potential health risks [115].

In yet another global antimicrobial surveillance study, surveillance data reveal that 20–30 % of *Streptococcus pneumoniae* strains are resistant to macrolides. Among these resistant strains, about two-thirds exhibit resistance through an efflux mechanism, while the remaining third involve modification of the ribosomal target site by methylases. Additionally, *Haemophilus influenzae* strains, although less widely recognized for macrolide resistance, commonly possess intrinsic efflux pumps that reduce intracellular macrolide concentrations. These findings highlight the importance of cautious antibiotic use in paediatric populations to mitigate the development and spread of macrolide resistance [116].

Macrolides have been studied in various contexts, revealing both therapeutic benefits and risks in infants. In preterm low birth weight infants, erythromycin (as both prophylaxis and rescue therapy) was shown to reduce the time to achieve full enteral feeding and decrease hospitalization duration without increasing adverse events, based on randomized controlled trials [117]. However, in a cohort study, macrolide use, particularly within the first 120 days, was strongly associated with an increased risk of infantile hypertrophic pyloric stenosis (IHPS), underscoring the need for caution in prescribing [118]. Macrolides have also been explored in a meta-analysis for their role in reactive airway diseases (RADs) in children, where they improved pulmonary function, reduced rescue medication use, and decreased recurrent wheezing episodes [119]. In children with bronchiectasis, long-term macrolide use showed limited effectiveness in reducing pathogen presence, particularly for *Moraxella catarrhalis*, without significant effects on forced expiratory volume or adverse events [120]. Furthermore, a retrospective cohort study of 374 children with Mycoplasma pneumoniae pneumonia in 2023 revealed a high macrolide resistance rate of 87 %, along with prolonged fever and higher hospitalization rates despite the widespread use of macrolides in combination with corticosteroids [121]. This highlights the ongoing challenge of macrolide resistance in treating respiratory infections in children. These findings suggest the need for cautious and judicious use of antibiotics in early life to minimize potential harm and preserve the integrity of the gut microbiome. Further research is essential to fully understand the implications of these changes and to develop strategies to mitigate their adverse effects.

6.2. Macrolide impact on gut microbiota in adults

Exposure to macrolide antibiotics in adults can disrupt this established microbiota, leading to reduced microbial diversity and potential overgrowth of opportunistic pathogens [122]. However, the adult microbiota's resilience often allows it to recover more effectively than the infant microbiota, Studies have shown that antibiotic use in adults can cause temporary shifts in microbial composition, but the microbiota generally returns to its baseline state once antibiotic treatment is discontinued [123]. In a prospective observational study *Helicobacter pylori* eradication using reverse hybrid therapy demonstrated that while this treatment induces temporary gut dysbiosis, marked by significant fluctuations in the relative abundances of Firmicutes, Actinobacteria, and Proteobacteria, these changes were not permanent. Importantly, the erm (B) gene, which confers macrolide resistance, showed a transient increase post-treatment but eventually returned to baseline levels within a

year. This suggests that while macrolide resistance genes can be temporarily elevated following antibiotic treatment, they may not persist long-term [124].

In a cross-sectional study, 15 % of the stool samples from 100 healthy people included methicillin-susceptible *Staphylococcus aureus* (MSSA). Three of these isolates showed resistance to clindamycin and erythromycin, which was associated with the presence of the resistance genes Mph(C) + erm(A) and/or erm(C). This suggests that target site alteration and active efflux mechanisms are the methods by which these MSSA bacteria mediate their resistance to macrolides. The identification of these resistance genes highlights the possibility of macrolide resistance in healthy persons' non-pathogenic gut flora. Due to their potential to act as repositories for resistance genes that could spread to harmful strains, commensal bacteria emphasize the significance of tracking and comprehending trends of antibiotic resistance [125].

Additionally, a different study shows that *Bifidobacterium breve* CECT7263 has an uncommon level of resistance to the macrolides clindamycin and erythromycin. A genomic region with a gene homologous to rRNA methylase genes which are known to provide resistance to macrolides, was connected to the resistance phenotype. When this gene was inserted into *Escherichia coli*, it was able to impart erythromycin resistance. This resistance gene is extremely uncommon; it has only been found in one strain of *Bifidobacterium longum* and two strains of *Bifidobacterium breve*. Although the gene is placed within a putative genomic island, suggesting that it may have been acquired through horizontal gene transfer, in vitro conjugation assays did not show gene transfer to other bacteria. This finding highlights the potential for bifidobacteria to act as reservoirs of macrolide resistance genes, which could pose a risk for transferring resistance to intestinal pathogens [126].

The effects of macrolides on the adult gut microbiota differ significantly between acute and chronic use. Acute administration, such as a short course of macrolides, typically results in transient disruptions. For instance, Clarithromycin reduced the populations and diversity of Enterobacteria as well as the anaerobic bacteria *Bifidobacterium* sp and *Lactobacillus* sp, with these effects lasting up to five weeks [123]. Further, studies have shown that while there is an immediate drop in microbial diversity and changes in the gut flora composition, these are often reversible. The gut microbiota usually returns to its baseline state after the antibiotic course ends, though the recovery may be incomplete, and resistant strains and opportunistic pathogens may temporarily thrive. Azithromycin specifically causes more alterations in the composition of the microbiome by delaying the recovery of species richness [99]. Following the administration of antibiotics, some people encounter a long-lasting decrease in microbiome diversity, with their microbiome profiles mirroring those of patients in intensive care units [99]. These observations enhance our understanding of how antibiotics affect the commensal microbiome's dynamics, resilience, and recovery. Chronic use of macrolides and its prolonged exposure maintain selective pressure that favours resistant strains. Macrolides, for example, have been shown to dramatically change the gut microbiota by decreasing the abundance of *Enterococcus* sp. and *E. coli* and increasing the abundance of Enterobacteriaceae, such as *Citrobacter* sp., *Enterobacter* sp, and *Klebsiella* sp. Additionally, they significantly reduced the amount of anaerobic bacteria. The macrolide treatment caused alterations in the microbiota that persisted for up to four years when metronidazole and clarithromycin were combined [110]. This prolonged use can lead to a persistent dysbiotic state.

6.3. Impact of polypharmacy and macrolides on gut microbiota in the elderly

Polypharmacy, the simultaneous use of multiple medications commonly seen in the elderly, including macrolide antibiotics, can greatly affect the gut microbiota [127] In a study, metagenomics reveals distinct impacts of various medications on the human gut microbiome. the most prevalent ARGs were those targeting tetracyclines and macrolides, and those connected to the resistance-nodulation-cell division superfamily of efflux pumps and the main facilitator superfamily [128]. The elderly population often faces chronic health conditions that necessitate the use of various medications, leading to complex drug regimens [127]. Macrolides, such as erythromycin, azithromycin, and clarithromycin, are frequently prescribed in this demographic for respiratory infections, skin infections, and other bacterial ailments [129]. Macrolides are recognized for their potential to induce gastrointestinal intolerance, liver toxicity, heart issues, central nervous system toxicity and hearing problems Therefore, their judicial use is advised in the elderly [127,129–131].

The impact of polypharmacy on the gut microbiota is multifaceted. Firstly, antibiotics like macrolides can disrupt the balance of the gut microbiota by eliminating both pathogenic and beneficial bacteria, leading to a decrease in microbial diversity [132]. This disruption is particularly concerning in the elderly, who already experience a natural decline in gut microbiota diversity with age [133]. Reduced microbial diversity is associated with increased susceptibility to infections, metabolic disorders, and inflammatory conditions [133].

Additionally, polypharmacy, including the use of macrolides, can promote the growth of antibiotic-resistant bacteria in the gut [128]. As a result, antibiotic-resistant strain may persist and spread within the gut microbiota, posing a risk for infections that are challenging to treat. Prolonged or repeated antibiotic exposure can lead to the enrichment of resistant bacteria harbouring resistance genes, such as those encoding for macrolide resistance mechanisms [134]. for instance, in a study spanning 20 years in northeastern China, 1240 erythromycin-resistant *Streptococcus pneumoniae* (ERSP) strains were analysed, revealing significant implications for macrolide resistance. Among these strains, nearly all (99.03 %) exhibited resistance to macrolides, lincosamides, and streptogramin B (MLSB phenotype), predominantly due to the presence of the erm(B) gene. This resistance profile was consistent across various age groups, highlighting widespread resistance among invasive and non-invasive isolates. Moreover, strains carrying both erm (B) and mef(A) genes showed multidrug resistance, emphasize the challenge in using macrolides for empirical treatment in elderly patients vulnerable to resistant pneumococcal infection [135]. Moreover, polypharmacy in the elderly can exacerbate gastrointestinal symptoms [136]. These symptoms not only affect quality of life but also contribute to further complications, including dehydration and nutrient malabsorption. Studies reveal Managing gastrointestinal diseases in the elderly is challenging due to comorbidities, polypharmacy, and limited life expectancy. Elderly patients often present with atypical, subtle symptoms, making timely diagnosis difficult. Polypharmacy and medication side effects complicate the clinical picture and can mislead treatment. These factors, along with comorbidities, increase the risk of complications. It is crucial to discuss care goals with elderly patients to ensure diagnostic and therapeutic interventions meet their expectations [136]. Thus, managing polypharmacy in this vulnerable population requires careful consideration of the potential impacts on gut health and microbiota composition, with a focus on optimizing therapeutic outcomes while minimizing adverse effects on gut microbial communities.

7. Recovery of gut microbiota following macrolide antibiotic discontinuation

The restoration of the gut microbiota after discontinuing macrolide antibiotics is a multifaceted process influenced by individual-specific factors, including the baseline microbial composition, the functional capacities of bacterial species, and external variables such as antibiotic regimens and lifestyle [137]. The dynamics of recovery differ substantially among individuals, with some experiencing rapid reestablishment of microbiota diversity and functionality, while others face prolonged dysbiosis [138].

7.1. Mechanisms facilitating microbiota recovery

Recovery-Associated Bacteria (RABs) are pivotal in restoring gut microbiota diversity and functionality post-antibiotic treatment. These species exhibit enhanced carbohydrate-degrading capabilities due to a higher abundance of carbohydrate-active enzymes, enabling efficient breakdown of complex carbohydrates from diet and host mucins [139]. RABs, such as *Bacteroides thetaiotaomicron*, *Bifidobacterium adolescentis, Akkermansia muciniphila and Bacteroides uniformis*, play significant roles in early-stage restoration [140,141]. The carbohydrate degradation by RABs supports increased microbial growth rates, with studies linking successful recovery to higher community growth driven by RAB activity. Additionally, RABs serve as primary colonizers, fostering ecological interactions that support the recolonization of other gut bacteria [142].

The metabolic activity of RABs produces SCFAs, which enhance gut health by stimulating mucin production, creating a positive feedback loop that accelerates recovery [141]. Machine learning models leveraging pre-treatment microbial abundance data have shown promise in predicting recovery outcomes and guiding targeted interventions [143,144].

7.2. Inter-individual variability in microbiota recovery

Significant heterogeneity exists in individual recovery trajectories post-antibiotic treatment. While some individuals demonstrate complete restoration of their microbiota to pre-treatment states, others experience long-term shifts in microbial composition and function. This variability is shaped by individual-specific microbiome profiles and genetic predispositions, highlighting the complexity of microbiota recovery processes [145]. Additionally, factors such as the type of antibiotic, duration of therapy, dosage, and combinations used markedly influence recovery patterns. Demographics, diet, and other lifestyle factors further modulate these outcomes, emphasizing the need for tailored approaches to mitigate dysbiosis [146].

7.3. Role of biomarkers and predictive tools

Advancements in microbiome research have led to the development of biomarkers capable of assessing post-antibiotic dysbiosis. For instance, the Microbiome Health Index for Antibiotic-induced Dysbiosis (MHI-A) has been proposed as a tool to differentiate dysbiotic states from healthy microbiota profiles. Such biomarkers provide insights into the extent of disruption and recovery, facilitating the design of targeted therapeutic interventions [147].

7.4. Implications for personalized recovery strategies

While the gut microbiota demonstrates resilience to antibiotic-induced perturbations, the speed and extent of recovery are highly variable [148]. This highlights the importance of developing personalized recovery strategies that leverage predictive biomarkers and focus on the introduction or stimulation of specific bacterial species associated with successful recovery. By integrating microbiome-targeted therapies with individualized clinical management, it is possible to mitigate the long-term consequences of antibiotic use and support microbiota resilience [149,150].

8. Various factors contributing to macrolide resistance development

Macrolide resistance development in the gut is influenced by a combination of factors, including antibiotic overuse, horizontal gene transfer, diet and lifestyle factors, and environmental contaminants. Understanding these factors is crucial for developing strategies to mitigate the spread of resistance and maintain the efficacy of macrolide antibiotics.

Antibiotic overuse remains the primary catalyst for the emergence of antibiotic resistance which is a critical medical challenge [134]. Positive selection for erm(F) and the development of resistance were seen at concentrations 1-2 orders of magnitude higher (>500 and < 750 μg/L) than average environmental values in a study looking at minimal selective concentrations for macrolides. Highlights the risk of resistance development due to antibiotic misuse in macrolides, posing potential threats to both human health and environmental stability [151]. Genes linked to antibiotic resistance, which have the potential to transfer to pathogens, can also be identified in beneficial bifidobacteria species [152]. In a randomized controlled trial, macrolides when used or prescribed without appropriate consideration, create selective pressure on gut bacteria, facilitating the survival and proliferation of resistant strains. For instance, widespread administration of azithromycin to preschoolers in sub-Saharan Africa twice a year for two years reduced childhood mortality rates but concurrently elevated levels of macrolide resistance [153].

The gut is a hotspot for horizontal gene transfer due to the dense and diverse microbial community it harbours [154]. Resistance genes can be transferred between different bacterial species via plasmids, transposons, or integrons [155]. The discovery of the erm(51) gene in *Rhodococcus equi*, associated with transposons and plasmids, suggests that horizontal gene transfer is a significant mechanism for spreading macrolide resistance in environmental pathogens, likely driven by antimicrobial exposure [156]. Further, the Erm genes can be easily spread through HGT, leading to widespread resistance. This gene transfer is facilitated by the close proximity of bacteria in the gut environment, increasing the chances of gene exchange. For instance, Bifidobacteria, particularly *Bifidobacterium longum*, may act as reservoirs for the erythromycin resistance gene erm(X). Conjugation assays have demonstrated that erm(X) can transfer to multiple bifidobacterial strains, highlighting the potential risk of ARG transfer to pathogens [152]. These resistance genes' identification highlights the possibility of macrolide resistance even in healthy people's non-pathogenic gut microbiota. Given that commensal bacteria might act as repositories for resistance genes that may spread to pathogenic strains, it is critical to keep an eye on and comprehend patterns of antibiotic resistance in these bacteria [125].

Diet plays a significant role in shaping the gut microbiome and can influence the development of antibiotic resistance [157]. Additionally, consumption of animal products containing residual antibiotics can introduce resistant bacteria into the human gut. For example, the investigation of three Lactobacillus strains isolated from chicken meat shows that these bacteria have the ability to transmit ARGs to pathogenic strains. In particular, it was found that the macrolide resistance gene erm(B) transferred both in vivo and in vitro. In vitro and animal models (Albino Wistar rats) were used to introduce *Lactobacillus salivarius* and *Lactobacillus reuteri* strains expressing erm(B). Stable transconjugants containing erm(B) were found in the recipient *Enterococcus faecalis* strains. Moreover, the transfer of tetracycline and macrolide resistance genes to pathogenic organisms such as Listeria monocytogenes and *Yersinia enterocolitica* was seen during food fermentation processes like fermented milk, idli batter, or chicken sausage [158]. These findings highlight the risk of macrolide resistance gene dissemination from foodborne *Lactobacillus* to human pathogens through the food chain. The study emphasizes the importance of monitoring and controlling ARG transfer in food processing environments to mitigate the potential spread of antibiotic resistance to humans via food consumption. Moreover, diets heavy in processed foods and poor in fiber might upset the balance of microbes in the gut, increasing the risk of resistant bacteria colonizing the microbiome [159]. Lifestyle factors such as stress can also negatively impact gut health and promote the growth of resistant bacteria. The symbiotic relationship is beneficial to both commensal microorganisms and their hosts. But stress does decrease Lactobacilli numbers. Conversely, gram-negative bacteria including *E. coli* and *Pseudomonas* exhibit enhanced proliferation,

epithelial adhesion, and mucosal uptake. Additionally, intestinal bacteria are able to detect when a host is under stress and when the chance arises, they increase their virulence factors [160].

Hospitalized patients are often exposed to broad-spectrum antibiotics, including macrolides, which can disrupt their gut microbiota and select for resistant strains. According to the cross-sectional study, 67.9 % of hospital-acquired Enterococci had the cMLSB (constitutive MLSB) phenotype, which is a prominent resistance pattern among enterococcal isolates. The cMLSB phenotype is encoded by the erm(B) gene, which was found to be predominant in 33.3 % of isolates obtained from hospitals. Furthermore, a significant MLSB resistance factor, the lsa(A) gene, was discovered in 33.3 % of isolates obtained in hospitals. These results suggest that Enterococci in hospital settings possess notable MLS resistance, namely the cMLSb phenotype facilitated by the erm(B) and lsa(A) genes [161]. Also, a cross-sectional study conducted at Okayama University Hospital reveals a noteworthy incidence of inducible MLSB (iMSLB) resistance among isolates of *Staphylococcus aureus* that are susceptible to clindamycin. This resistance phenotype, primarily mediated by the erm(A) gene, was more prevalent in methicillin-resistant *S. aureus* (MRSA) compared to MSSA. These findings bring out the importance of monitoring and managing nosocomial transmission of iMLSB-resistant *S. aureus* strains, particularly among vulnerable patient populations in hospital settings [162]. This emphasizes the significance of monitoring and controlling macrolide resistance in nosocomial settings.

The development of resistance in the gut can be facilitated by the presence of antibiotics and antibiotic-resistant bacteria in the environment, especially in food products, agricultural soils, and water supplies [163]. The increasing prevalence of antibiotic-resistant pathogens in line numb*Staphylococcus aureus*, poses a significant zoonotic risk to human health. Antibiotic resistance developed in these pathogens in animals can transfer to humans through the food chain, leading to challenging infections that are difficult to treat with standard antibiotics [164]. Contaminated water sources used for drinking, irrigation, or recreational purposes can also introduce resistant bacteria into the human gut. Azithromycin-resistant bacteria isolated from the polluted Sava river sediments showed higher frequencies of erm(B), esr(E), Mph (E), and erm(F) compared to upstream sites. This indicates potential contamination of environmental bacteria with clinically relevant resistance genes, highlighting the risk of environmental sources contributing to the spread of macrolide resistance to human pathogens [165]. Understanding the multifaceted factors contributing to macrolide resistance development is crucial for developing effective strategies to combat this growing threat.

9. Strategies to mitigate macrolide resistance

The development and implementation of multifaceted strategies to combat the significant threat posed to public health by the emergence of macrolide resistance among common pathogenic bacteria, such as *Staphylococcus aureus, Bordetella pertussis, Mycoplasma pneumoniae*, and *Streptococcus pneumoniae* [166]. One promising approach involves the development of new derivatives that bypass existing resistance mechanisms. This can be achieved through a rational prioritization strategy for macrolide antibiotics, where candidates are screened based on solubility, membrane permeability, and binding affinity using free energy simulations and quantum mechanics/molecular mechanics calculations. After computational prioritization, the best candidates undergo experimental evaluation, resulting in a substance library highly enriched in compounds with antibacterial activity, allowing for faster iterations in developing new antibiotic derivatives [167].

Probiotic treatment can be a valuable approach to counteract the dysbiosis induced by macrolides, which disrupt the gut microbiota and impair immune function [168]. The reintroduction of beneficial microbial species through probiotics helps restore the balance of the intestinal microbiota, promoting mucosal immune system modulation and enhancing the host's well-being [169]. Their consumption can mitigate the negative effects of macrolide-induced dysbiosis, suppress immunopathology, and support intestinal immune as detailed in Table 1.

Another effective strategy involves the use of metabolomics to enhance the efficacy of existing antibiotics. For instance, exogenous L-methionine has been shown to restore the bactericidal activity of macrolides, doxycycline, and ciprofloxacin in multidrug-resistant *Streptococcus suis*. L-methionine affects methionine metabolism by reducing S-adenosylmethionine synthetase activity and decreasing methylation levels and efflux pump gene expression. This reduces the survival of *Streptococcus suis* by affecting oxidative stress and metal starvation, providing a new perspective on mitigating drug resistance in this pathogen [178]. Additionally, using antibiotic adjuvants presents a promising method to combat multidrug-resistant pathogens. Polymyxin B nonapeptide (PMBN), for instance, has demonstrated a synergistic effect with azithromycin in re-sensitizing *Escherichia coli* strains resistant to macrolides. PMBN increases the permeability of the bacterial outer membrane to azithromycin, significantly reducing bacterial growth and demonstrating morphological alterations in treated bacteria. This combination therapy represents a viable strategy for overcoming macrolide resistance [179].

The development of new macrolide derivatives that can bypass existing resistance mechanisms. Like, nafithromycin, a novel lactone ketolide, has shown potent in vitro activity against highly resistant *Streptococcus pneumoniae* isolates in both China and India. Nafithromycin effectively overcomes macrolide resistance mediated by erm and mef genes, demonstrating significant promise for treating community-acquired bacterial pneumonia [180,181]. Another strategy highlights combination therapies to enhance the efficacy of existing antibiotics. Hygromycin A, when used in combination with macrolides, significantly slows down the dissociation of macrolides from the ribosome and enhances their antimicrobial activity against macrolide-resistant bacteria. This synergistic interaction is particularly effective against strains expressing erm genes, providing a structural basis for overcoming erm-type resistance [182]. Additionally, combining rifabutin with clarithromycin has shown synergy against *Mycobacterium abscessus* strains harbouring the erm41 gene, by inhibiting the transcriptional induction of the whiB7–erm41 resistance system. This approach effectively suppresses inducible clarithromycin resistance [183].

Nanomedicine also holds great potential in addressing macrolide resistance. Encapsulating azithromycin in biocompatible polymers, such as poly (lactic-co-glycolic acid) (PLGA), has been shown to overcome efflux-resistant mechanisms in bacteria like MRSA and *Enterococcus faecalis*. The use of AZI-PLGA nanoparticles significantly reduced the minimum inhibitory concentration of azithromycin, highlighting the potential of polymer nanoparticles to improve the antibacterial effect of macrolides and tackle resistance issues [184]. Similarly, an innovative silver-zinc oxide nano-composite, when combined with erythromycin, showed significant antibacterial activity against MRSA and *Escherichia coli*. Notably, this nano complex exhibited approximately 20-fold reduced likelihood for developing bacterial resistance compared to erythromycin alone [185]. Thus, nanomedicine is a promising strategy against multidrug-resistant bacteria [186].

10. Future perspective

Advancing our understanding of macrolide-induced dysbiosis necessitates comprehensive research focusing on the long-term impacts across different age groups, especially infants and the elderly, who are particularly susceptible to microbiota disruptions. Employing advanced metagenomic, transcriptomic, and metabolomic techniques will provide granular insights into the specific bacterial taxa affected by macrolides and elucidate the functional and metabolic consequences of these alterations.

Future studies should prioritize the development of narrow-spectrum

Table 1
Impact of probiotics on microbiota composition during macrolide antibiotic treatment.

Probiotic Type	Macrolide Type	Study Type	Results on Microbiota	Reference
Lactobacillus rhamnosus SP1	Azithromycin	Triple-blind placebo-controlled, randomized clinical trial	No significant difference in microbiota composition between probiotics and azithromycin groups. Probiotics did not provide additional benefits in terms of modulating the gut microbiota compared to azithromycin or placebo.	[170]
Saccharomyces boulardii (supplementary regimens)	Clarithromycin, Amoxicillin (anti-*Helicobacter pylori* concomitant therapy)	Randomized, double-blind, placebo-controlled trial	- Significant alterations in gut and throat microbiota after treatment. - Most changes in gut microbiota reverted by Day 71. - Persistent alterations in throat microbiota. - Increased antibiotic resistance in *Enterobacteriaceae*, *Enterococcus* spp., and *Bacteroides* spp. - Probiotics led to stabilization of throat microbiota and reduced disturbances.	[171]
Saccharomyces boulardii	Azithromycin	Double-blind randomized placebo-controlled trial	*Saccharomyces boulardii* significantly reduced the duration and severity of diarrhea in children with acute colitis when co-administered with azithromycin. The intervention group showed lower Vesikari scores, fewer diarrhea episodes, and reduced duration of diarrhea, fever, and hospitalization compared to the control group.	[172]
Probiotic Combination (*Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freudenreichii* ssp. shermanii JS, *Bifidobacterium breve* Bb99)	Clarithromycin, Amoxicillin	Randomized controlled trial	The probiotic combination slightly counteracted treatment-induced alterations in the intestinal microbiota, reducing alterations in the total numbers of aerobes and *lactobacilli/enterococci*. The anti-*H. pylori* treatment induced long-term disturbances in microbiota, but probiotics resulted in minor changes.	[173]
Saccharomyces boulardii (EMYA-01)	Erythromycin	In vitro and in vivo study (animal study)	*Saccharomyces boulardii* EMYA-01 removed 71.3 ± 1.6 % of erythromycin from fecal suspension in vitro. In vivo, it reduced up to 40 % of free antibiotics in the gut of erythromycin-treated mice without affecting antibiotic distribution in targeted organs. EMYA-01 restored intestinal microbiome diversity and mitigated antibiotic-induced dysbiosis.	[174]
Bifidobacterium longum (BB536) and *Lentinula edodes* mycelia (AHCC®)	Azithromycin	Double-blind randomized clinical trial	BB536 and AHCC® co-administration with azithromycin enhanced anti-inflammatory responses by modulating T regulatory cell and dendritic cell (DC) phenotypes. Foxp3 expression increased in volunteers receiving BB536, and combination treatment increased myeloid dendritic cells (mDC) and mDC2 phenotypes.	[175]
Clostridium butyricum plus *Bifidobacterium infantis*	Azithromycin	Randomized clinical trial	No cases of antibiotic-associated diarrhea occurred in children. Probiotics partially restored gut microbiota, improved intestinal mucosal barrier function, and reduced systemic inflammation (interleukin 10). Probiotic co-administration with azithromycin is a promising therapy for preventing and treating antibiotic-associated diarrhea.	[176]
Lactulose (prebiotic)	Azithromycin	16S rRNA gene sequencing analysis, observational study	Azithromycin caused an increase in opportunistic pathogens like *Streptococcus*. Lactulose promoted saccharolytic bacteria such as *Anaerostipes, Blautia, Lactobacillus, Enterococcus,* and *Roseburia*, providing protection against pathogens. The combination restored the microbiome closer to pre-treatment state.	[177]

antibiotics and macrolide derivatives that selectively target pathogenic bacteria while sparing commensal gut microbiota. Bioconjugation techniques offer promising avenues to enhance antibiotic specificity and efficacy, potentially reducing off-target effects on the microbiota. Additionally, nanomedicine approaches could revolutionize antibiotic delivery, allowing for targeted release and minimizing systemic exposure, thereby preserving gut microbiota diversity.

Exploring the potential of herbal medicines as adjunct therapies is another promising direction. Herbal compounds have shown potential in modulating gut microbiota composition and enhancing host immune responses, which could mitigate macrolide-induced dysbiosis. Rigorous clinical trials are warranted to evaluate the safety and efficacy of these approaches in clinical settings.

Moreover, personalized medicine strategies leveraging individual microbiota profiles could optimize antibiotic regimens, thereby minimizing adverse effects and resistance development. Enhancing antibiotic stewardship programs is critical to ensure the judicious use of macrolides, particularly in vulnerable populations.

Interdisciplinary collaboration, integrating insights from microbiology, pharmacology, clinical medicine, and public health, is essential to develop innovative strategies that safeguard gut microbiota integrity and mitigate the burgeoning issue of antibiotic resistance. By fostering such collaborative efforts, we can enhance therapeutic outcomes and maintain the delicate balance of the human microbiome amidst widespread antibiotic use.

CRediT authorship contribution statement

H. Shayista: Writing – original draft, Resources. **M.N. Nagendra Prasad:** Data curation. **S. Niranjan Raj:** Conceptualization. **Ashwini Prasad:** Data curation. **S. Satish:** Data curation. **H.K. Ranjini:** Methodology. **K. Manju:** Methodology. **Ravikumara:** Methodology. **Raghuraj Singh Chouhan:** Writing – review & editing. **Olga Y. Khohlova:** Writing – review & editing. **Olga V. Perianova:** Writing – review & editing. **S. Lakshmi:** Methodology. **Syed Baker:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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