

# Antibiotic Resistance via Horizontal Gene Transfer in Commensal Microbiota of Healthy Individuals with Self-Medication Practices: A Questionnaire-Based and In Vitro Study

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Received: 05/02/2026 | Accepted: 24/03/2026 | Published: 27/04/2026

## Abstract:

**Background:** Antibiotic resistance is a growing global health concern driven largely by inappropriate antibiotic use. Commensal microbiota of healthy individuals serve as an important reservoir of antibiotic resistance genes, which can be disseminated through horizontal gene transfer (HGT). Self-medication with antibiotics further accelerates this process by exerting selective pressure on microbial populations.

**Aim:** To assess antibiotic resistance mediated by horizontal gene transfer in commensal microbiota of healthy individuals with self-medication practices using a questionnaire-based survey and in vitro analysis.

**Materials and Methods:** This cross-sectional study was conducted over a defined period among healthy individuals attending Nandha Siddha Medical College and Hospital. A structured questionnaire was used to collect data on antibiotic self-medication practices, including frequency, indications, and sources of antibiotic use. Biological samples (stool and/or throat swabs) were collected for isolation of commensal bacteria and transported to CML Biotech Ltd, Angamaly, Kerala, for microbiological screening and analysis. Isolates were identified using standard microbiological methods and subjected to antibiotic susceptibility testing using the Kirby–Bauer disc diffusion method as per CLSI guidelines. Selected resistant strains were further analyzed for horizontal gene transfer using in vitro conjugation experiments..

**Results:** A significant proportion of participants reported self-medication with antibiotics. Commensal bacterial isolates from self-medicated individuals showed a higher prevalence of antibiotic resistance compared to non-users. Multidrug-resistant strains were frequently observed among isolates from the self-medication group. In vitro conjugation studies demonstrated successful transfer of resistance determinants between donor and recipient strains, confirming the role of horizontal gene transfer in resistance dissemination. Statistical analysis showed a significant association between self-medication practices and the presence of resistant isolates ( $p < 0.05$ ).

**Conclusion:** The study highlights that self-medication with antibiotics contributes significantly to the emergence of antibiotic resistance in commensal microbiota. Horizontal gene transfer plays a crucial role in the dissemination of resistance genes, emphasizing the need for strict antibiotic stewardship and public awareness programs to curb indiscriminate antibiotic use.

**Keywords:** Antibiotic resistance, horizontal gene transfer, commensal microbiota, self-medication, multidrug resistance, resistome.

## Introduction

Antibiotic resistance has emerged as one of the most pressing global public health challenges, threatening the effective prevention and treatment of infectious diseases. The widespread and often inappropriate use of antibiotics has accelerated the development of resistant microorganisms, reducing the efficacy of commonly used drugs and increasing morbidity, mortality, and healthcare costs [1]. While clinical settings have traditionally been the focus of antimicrobial resistance surveillance, growing attention is being directed toward the role of the community and apparently healthy individuals in the propagation of resistance.

The human body harbors a vast and diverse population of commensal microbiota, particularly within the gastrointestinal and respiratory tracts. These microorganisms, although generally harmless, serve as a significant reservoir of antibiotic resistance genes, collectively referred to as the “resistome” [2]. Under selective pressure from antibiotic exposure, even non-pathogenic bacteria can acquire and maintain resistance traits. These traits may later be transferred to pathogenic organisms, contributing to the emergence of difficult-to-treat infections.

Horizontal gene transfer plays a crucial role in the dissemination of antibiotic resistance among bacteria. Mechanisms such as transformation, transduction, and conjugation enable the exchange of genetic material, including plasmids and transposons carrying resistance determinants, between bacterial species [3]. This genetic exchange can occur within the commensal flora of healthy individuals, facilitating the silent spread of resistance even in the absence of overt infection or hospitalization.

Self-medication with antibiotics, particularly in regions where over-the-counter access is common, is a major driver of antimicrobial resistance. Individuals often consume antibiotics without proper medical guidance, inappropriate dosing, or incomplete courses, creating an ideal environment for the selection and propagation of resistant strains [4]. Such practices not only affect the individual but also contribute to the broader community reservoir of resistance genes, which can be transmitted through direct or indirect contact.

Given the increasing concern regarding community-driven antibiotic resistance, it is essential to investigate the relationship between self-medication practices and the development of resistance within commensal microbiota. This study aims to assess the presence and transfer of antibiotic resistance among commensal bacteria in healthy individuals who practice self-medication, using a combination of questionnaire-based data collection and *in vitro* analysis. The objectives are to evaluate patterns of antibiotic self-use, identify the prevalence of resistance in commensal isolates, and examine the role of horizontal gene transfer in the dissemination of resistance traits.

## Materials and Methods

This study was designed as a cross-sectional, questionnaire-based and *in vitro* analytical study conducted among healthy individuals to evaluate the role of self-medication with antibiotics in the development and transfer of antimicrobial resistance in commensal microbiota. The study was conducted at Nandha Siddha Medical College and Hospital over a period of six months, from June 2025 to November 2025. Microbiological screening and laboratory testing were carried out at CML Biotech Ltd, Angamaly, Kerala.

Informed consent was obtained from all participants prior to enrollment.

A total of 100 healthy individuals aged between 18–40 years with no history of recent hospitalization or active infection were included in the study. Participants were selected using convenience sampling. Individuals who had received prescribed antibiotics under medical supervision in the past one month or had chronic illnesses were excluded. Data regarding antibiotic self-medication practices, including frequency, duration, type of antibiotics used, and source of procurement, were collected using a pre-validated structured questionnaire.

Samples were collected from each participant to isolate commensal bacteria. Depending on feasibility, stool samples and/or throat swabs were obtained under aseptic conditions and transported promptly to the microbiology laboratory at CML Biotech Ltd, Angamaly, Kerala, for processing. The samples were cultured on appropriate media such as MacConkey agar, blood agar, and nutrient agar, followed by incubation at 37°C for 18–24 hours. Bacterial isolates were identified based on colony morphology, Gram staining, and standard biochemical tests.

Antibiotic susceptibility testing of the isolates was performed using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Commonly used antibiotics such as ampicillin, ciprofloxacin, tetracycline, and cephalosporins were tested. The presence of multidrug resistance was noted and recorded.

To assess horizontal gene transfer, selected resistant isolates were subjected to *in vitro* conjugation experiments. Donor strains exhibiting antibiotic resistance were mixed with susceptible recipient strains under controlled laboratory conditions at CML Biotech Ltd.

After incubation, transconjugants were selected on antibiotic-containing media, and the transfer of resistance traits was confirmed by observing growth patterns and repeating antibiotic susceptibility testing.

Data obtained from the questionnaire and laboratory findings were entered into a database and analyzed using appropriate statistical methods. Descriptive statistics such as percentages and proportions were used to summarize self-medication practices and resistance patterns. Associations between antibiotic self-medication and presence of resistance were evaluated using chi-square test, with a *p*-value of <0.05 considered statistically significant.

## Results

### Demographic Characteristics of Study Participants

A total of 100 healthy individuals were included in the study. The majority of participants belonged to the age group of 20–30 years, with a slight male predominance. All participants were asymptomatic at the time of sample collection and had no recent history of hospitalization or active infection.

### Questionnaire-Based Assessment of Antibiotic Self-Medication

The analysis of questionnaire responses revealed that a significant proportion of participants practiced antibiotic self-medication.

- **Prevalence of self-medication:** 64% of participants reported using antibiotics without a prescription.

- **Common indications:** Fever (40%), sore throat (25%), cough/cold (20%), and dental pain (15%).
- **Source of antibiotics:** Local pharmacies (70%), previous prescriptions (20%), and advice from friends/family (10%).
- **Completion of antibiotic course:** Only 45% completed the full course, while 55% discontinued once symptoms improved.

**Table 1: Distribution of participants based on self-medication practices**

Practice	Number (n=100)	Percentage (%)
Self-medication present	64	64%
No self-medication	36	36%

**Table 2: Indications for antibiotic self-medication**

Indication	Number	Percentage (%)
Fever	40	40%
Sore throat	25	25%
Cough/Cold	20	20%
Dental pain	15	15%

### Isolation and Identification of Commensal Bacteria

Commensal bacteria were successfully isolated from all samples. The commonly isolated organisms included *Escherichia coli*, *Staphylococcus spp.*, and *Klebsiella spp.* Identification was based on colony morphology, Gram staining, and biochemical characteristics.

### Antibiotic Susceptibility Pattern

Antibiotic susceptibility testing revealed that a considerable proportion of isolates exhibited resistance to commonly used antibiotics. Resistance was most frequently observed against ampicillin and tetracycline, while relatively lower resistance was noted for higher-generation antibiotics.

- **Multidrug resistance (MDR):** Observed in 38% of isolates
- Higher resistance rates were noted among isolates from individuals practicing self-medication

**Table 3: Antibiotic resistance pattern among isolates**

Antibiotic	Resistant Isolates (%)
Ampicillin	60%
Tetracycline	52%
Ciprofloxacin	35%
Cephalosporins	28%

### Association between Self-Medication and Antibiotic Resistance

A significant association was found between antibiotic self-medication and the presence of resistant commensal bacteria. Among participants practicing self-medication, 70% showed resistant isolates compared to 30% among non-users.

Statistical analysis using the chi-square test showed a **p-value < 0.05**, indicating a significant correlation between self-medication and antibiotic resistance.

**Table 4: Association between self-medication and resistance**

Category	Resistant (%)	Non-resistant (%)
Self-medication (n=64)	70%	30%
No self-medication (n=36)	30%	70%

### In Vitro Demonstration of Horizontal Gene Transfer

In vitro conjugation experiments demonstrated successful transfer of antibiotic resistance from donor to recipient strains. Growth of transconjugants on selective media confirmed the acquisition of resistance traits. This supports the role of horizontal gene transfer in the dissemination of resistance among commensal bacteria.

The study demonstrates a high prevalence of antibiotic self-medication among healthy individuals, with a significant proportion harboring resistant commensal bacteria. The statistically significant association ( $p < 0.05$ ) and successful in vitro gene transfer experiments highlight the role of self-medication in promoting antibiotic resistance through horizontal gene transfer.

### Discussion

The present study evaluated antibiotic resistance among commensal microbiota in healthy individuals with a history of self-medication and demonstrated a significant association between non-prescribed antibiotic use and increased antimicrobial resistance. The findings also highlight the role of horizontal gene transfer (HGT) as a key mechanism driving dissemination of resistance genes within the human microbiome.

In this study, 64% of participants reported self-medication with antibiotics, which is comparable to other community-based studies reporting high prevalence of non-prescription antibiotic use ranging from 40% to 70% in developing countries [5,6]. Similar patterns were observed in studies by Ocan et al., who reported widespread self-medication practices contributing significantly to antimicrobial resistance burden in community settings [7]. This suggests that unrestricted antibiotic access remains a major global public health concern.

The present study demonstrated that 70% of individuals practicing self-medication harbored antibiotic-resistant commensal bacteria compared to only 30% among non-users. A similar association has been reported in recent metagenomic studies where gut microbiota of healthy individuals served as a major reservoir of antibiotic resistance genes (resistome) [8]. Sommer et al. also emphasized that commensal bacteria act as silent carriers of resistance determinants that can be transferred to pathogenic organisms [9].

**Table 5: Comparison of self-medication and resistance prevalence with recent studies**

Study	Population	Self-medication (%)	Resistance in commensals (%)	Key finding
Present study	Healthy individuals	64	70 (self-medicated group)	Significant association (p<0.05)
Ocan et al. [7]	Community population	50–75	High prevalence	Antibiotic misuse linked to AMR
Sommer et al. [9]	Gut microbiome studies	—	High resistome load	Commensals act as resistance reservoir
Van Schaik et al. [10]	Microbiome analysis	—	Variable	HGT drives resistance spread

Horizontal gene transfer plays a central role in the dissemination of antibiotic resistance genes in commensal flora. In the present study, in vitro conjugation experiments confirmed transfer of resistance traits between bacterial strains. This is consistent with recent evidence showing that conjugation, transformation, and transduction facilitate rapid spread of resistance genes within the gut microbiome [11]. Studies have demonstrated that antibiotic exposure significantly increases the rate of HGT, thereby enhancing resistance acquisition [12].

Recent literature also highlights that antibiotic pressure increases resistome diversity and promotes genetic exchange among bacteria. Forslund et al. reported that short-term antibiotic exposure leads to increased copy numbers of resistance genes and enhanced gene transfer potential in gut bacteria [13]. Similarly, Zhang et al. demonstrated that commensal bacteria in healthy individuals frequently exchange resistance genes within and across species boundaries [14].

**Table 6: Mechanisms of horizontal gene transfer in resistance spread**

Mechanism	Description	Relevance in study
Conjugation	Plasmid transfer via cell contact	Observed in vitro
Transformation	Uptake of free DNA	Possible in gut flora
Transduction	Bacteriophage-mediated transfer	Reported in literature
Mobile genetic elements	Integrations, transposons	Major resistance carriers

The statistically significant association observed in this study (p < 0.05) aligns with global findings emphasizing the impact of antibiotic misuse on resistance development. Recent reviews suggest that inappropriate antibiotic consumption is a major driver of selection pressure, leading to survival and propagation of resistant strains within the human microbiome [15]. Furthermore, environmental and dietary exposures may also contribute to resistance gene selection and dissemination [16].

The findings of this study support the concept that the human gut acts as a dynamic reservoir of antimicrobial resistance genes. This is consistent with recent large-scale metagenomic studies showing that resistance genes are widely distributed even among healthy populations and are influenced by lifestyle, antibiotic exposure,

and microbial interactions [17]. The “One Health” approach emphasizes that human, animal, and environmental microbiomes are interconnected in the spread of resistance [18].

Overall, this study highlights that self-medication with antibiotics significantly contributes to the development and spread of antimicrobial resistance through horizontal gene transfer mechanisms in commensal microbiota. Strengthening antibiotic stewardship programs and public awareness is essential to reduce unnecessary antibiotic use and limit resistance propagation.

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