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The Antimicrobial Resistance Trend Pattern in A Subset Population of West Bengal

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Abstract

A deadly pandemic has threatened the world and is witnessing severe climate change. Constant environmental changes are leading to various viral/bacterial infections, which have increased the consumption of antibiotics. The rise in usage of antibiotics has increased significantly during the COVID-19 pandemic and post-COVID era. Generally, the most prescribed antibiotics fall under the broad-spectrum range as these work on many gram-positive and gram-negative disease-causing bacteria. There has been an increase in the accessibility and affordability of antibiotics among the population. The primordial factor for this is the over-the-counter buying of antibiotics followed by frequent usage of antibiotics. Patients often only finish part of the course of antibiotics. Frequent consumption of antibiotics and not completing the whole course of the antibiotics pose a severe and potentially irreversible threat to the healthy microbiome of the human intestine and a resistance pattern against the antibiotics. The study specifically focuses on understanding the antibiotic resistance pattern in the post-COVID scenario on the gut microbiome of the West Bengal population, making it particularly relevant to the local healthcare community. It underscores the urgent need for responsible antibiotic use, public awareness of the use of antibiotics, and antimicrobial resistance.

Keywords: *Antibiotics, Antibiotic Use in India, Antimicrobial Resistance, Bacteria, Gut Microbiome, Over-The-Counter.*

Introduction

The COVID-19 pandemic led to the death of many people and left behind a trail of recurrent infections in the survivors. Climate change causes fluctuations in temperature and seasonal changes, which are ideal causes of viral and bacterial infections. There is a high burden of different types of diseases. Human health is always at stake [1]. The rampant use of antibiotics in India is multifactorial. Antibiotics treat any infection [2]. Usually, the most prescribed antibiotics fall under the broad-spectrum range as these work on a vast range of disease-causing bacteria (gram-positive and gram-negative) [3]. Generally, bacterial infections are urinary tract infections (UTIs), throat infections (pharyngitis), pneumonia, ear infections (otitis media), sinus infections (sinusitis), and many more [4].

Antibiotics are medicines that hinder infections caused by bacteria by either killing the bacteria or keeping them from copying themselves or reproducing. The word antibiotic stands for “against life” [5].

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Scientists introduced the first antibiotic, salvarsan, in the 1900s. Antibiotics have changed medicine since then, adding about 23 years to the average human lifespan. Penicillin's discovery in 1928 marked the start of a golden era in finding natural antibiotics, peaking in the 1950s [5].

Antibiotics come in various forms, such as pills, injections, and topicals [5].

An antibiotic has either a narrow or broad spectrum of activity. Narrow-spectrum antibiotics are more specific and active only against certain bacteria groups or strains of bacteria [6].

Broad-spectrum antibiotics are a class of antibiotics that act against an extensive range of disease-causing bacteria by targeting both gram-positive and gram-negative bacterial groups. They are often grouped by their ability to act upon different bacterial groups [7].

Broad-spectrum antibiotics can treat a wide range of bacterial infections and conditions. They are designed to promote improved side effects management while simultaneously reducing the threat of hospital readmissions. Intravenous antibiotics also work, whereas oral antibiotics have failed [7].

Antibiotics primarily obstruct the essential processes or structures in the bacterial cell, killing the bacteria and slowing their growth. Depending on these effects, an antibiotic is considered bactericidal or bacteriostatic [6].

A bactericidal antibiotic kills the bacteria, whereas bacteriostatic antibiotics stop bacterial growth without killing them. The human immune system then needs to clear the infection. Figure 1 depicts the different classes of antibiotics and their mechanisms of action.

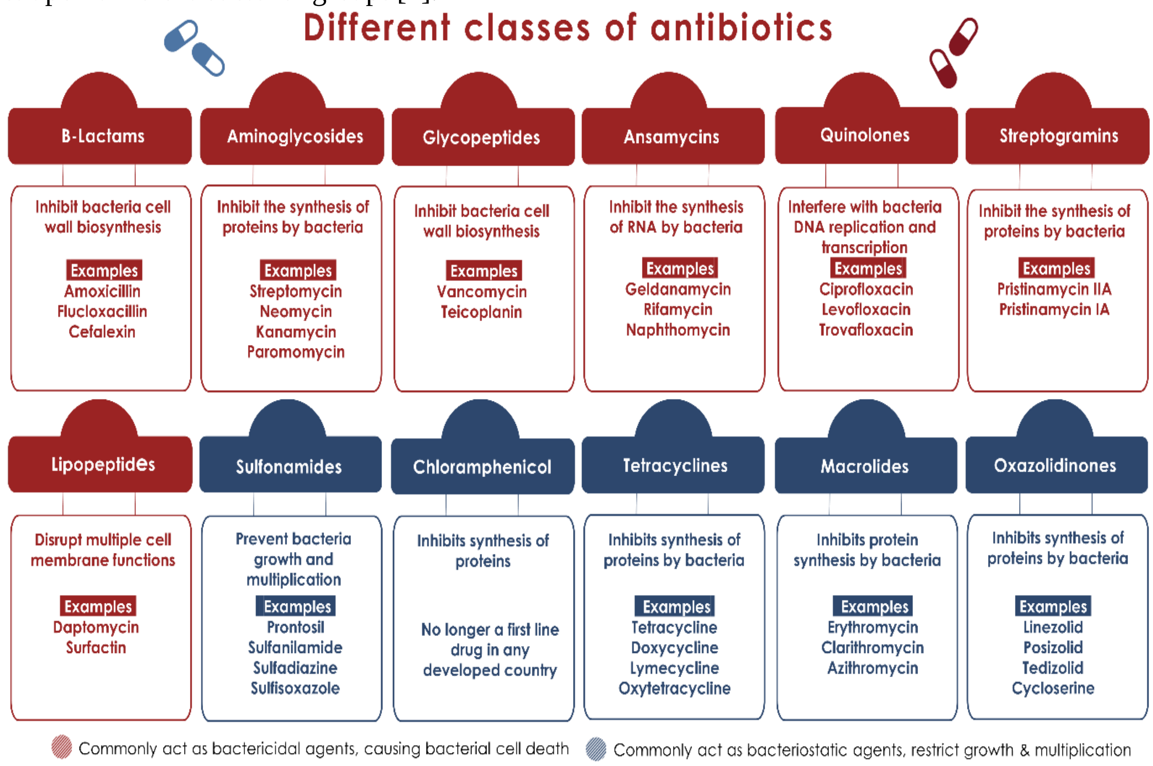


Figure 1. Types and Classes of Antibiotics

Several antibiotic classes have different action mechanisms and bacterial targets [6]. The three main antibiotic targets in bacteria:

1. The cell wall or membranes of the bacterial cell
2. The machineries that make the nucleic acids DNA and RNA
3. The machinery that produces proteins (the ribosome and associated proteins)

Broad-spectrum antibiotics bypass gut absorption, entering the bloodstream to deliver a speedy, more manageable way to restore your health to its best [7]. Antibiotic medicine impacts the gastrointestinal system, disrupting the gut microbiome [8,9]. The first signs of an unhealthy gut generally found during antibiotic consumption are irritable bowel syndrome and diarrhoea [8,10]. Antibiotics can have numerous negative impacts on the gut microbiota, from reduced diversity of species, alteration in metabolic activity, and the selection of antibiotic-resistant organisms, resulting in downstream effects such as antibiotic-associated diarrhoea and recurring *C.difficile* infections [11]. Early antibiotic exposure can impact gastrointestinal, immunological, and neurocognitive systems. This is problematic because of the increased use of antibiotics and indicates a future increase in the widespread presence of acute conditions [11].

Eventually, this leads to the development of multidrug-resistant organisms in the gut microbiome, thereby beginning antimicrobial resistance. Multidrug-resistant microorganisms have developed resistance to many antibiotics, making them difficult to treat.

Antimicrobial resistance makes the disease-causing parasites flourish even in the existence of the medication that once impacted their growth [12]. This leads to more complications in recovery, and in some extreme cases, the disease-causing pathogen can resist the presently available medicines [12].

In India, the practice of purchasing antibiotics from pharmacy shops without a proper prescription, although illegal, is widespread [13]. This over-the-counter (OTC) purchase results in unregulated sales of antibiotics, a recognized pathway for the origin of antimicrobial resistance (AMR). This severe public health challenge demands urgent regulatory responses. All drugs that come under Schedule H and Schedule X of the Drugs and Cosmetics Rules, 1945, legally require a prescription for their sale. All other drugs are "non-prescription drugs." Antimicrobial agents (amas) fall under Schedule H and H1. In India, the term OTC is not legally recognized [14]. The global and Indian scenario of antimicrobial resistance is alarming, with India topping the list of countries with the highest antibiotic consumption and AMR [15]. The exploitation of antibiotics is a severe issue that could potentially take us back to the dark ages when antibiotics were unavailable, underscoring the requirement for caution and responsible antibiotic use.

Antibiotic resistance is more severe in developing countries with an increased burden of infectious disease, and healthcare spending is low [16]. Pan-resistant Gram-negative bacterial infections, which do not have a single sensitive antibiotic, are becoming too familiar in India [16]. Lack of adequate regulations and failure to implement the rules are among the primary causes of the inappropriate use of antibiotics. The data from past research across countries revealed that clinicians often prescribe antibiotics when they are not required or prescribe the wrong antibiotics to patients. Usually, there are circumstances where pharmacy staff are not fully qualified to sell these medicines, or they sell them without prescriptions.

It is also common for patients to not complete the entire course of antibiotics. The patients will likely stop taking medicine when recovery signs are visible. These are all various forms of antibiotic abuse.

All these cumulative factors lead to AMR if patients consume the wrong drug or dosage and for the wrong duration [17].

With increasing AMR, especially antibacterial resistance in bacteria, common infections are becoming difficult to treat [17]. Due to the increase in AMR trends, the most frequently used antibiotics now lose their effectiveness in treating the disease, resulting in prolonged hospitalization, a rise in healthcare expenses, and loss of life (in severe cases) [1].

There is also a decline in the available options for effectively treating bacterial infections.

Materials and Methods

This study was conducted on 100 human volunteers aged 18 and above from Kolkata in West Bengal. The healthy human volunteers were adequately informed about the study, and their consent was duly obtained for participation. The volunteers were counselled not to consume antibiotics 48-72 hours before collecting their stool samples. The doctor at the clinic checked their vital parameters and history of antibiotic consumption. The fresh stool sample was used for antimicrobial analysis.

Antimicrobial Susceptibility Testing: The minimum inhibitory concentration (MIC) was used. This method relies on phenotypic identification of susceptibility, necessitating the following steps:

1. Preparation of a standardized inoculum from a bacterial culture.
   a. Selection of well-isolated colonies
   b. Creating a bacterial suspension (inoculum)
   c. Standardising the suspension using McFarland suspension
   d. Diluting bacterial suspension (only MIC method)
2. Inoculating bacterial suspension into one of the following:
   a. A specific growth medium (e.g., Mueller Hinton Agar)
   b. A MIC panels
3. Incubating plates (disk diffusion) or panels (MIC)
4. Measuring the zone of inhibition or reading the MIC panel
5. Interpreting AST results

The “direct colony suspension method” is generally used to prepare inoculum from colonies grown within 18 to 24 hours, while the “growth method” can be utilized by incubating the inoculated broth (with fast-growing bacteria) within 2 to 6 hours. The standard McFarland turbidity for the inoculum is typically 0.5.

Dilution of bacterial suspension (commonly 1:20) for MIC must occur within 15 minutes after preparing the standard inoculum. Saline is often used as a diluent for a small amount of inoculum to achieve a concentration of 5 × 10 colony-forming units (CFU) per millilitre. As the inoculum is carefully poured over the panel tray and transferred to the panel prongs, the final concentration is expected to be relatively consistent.

Before inoculating bacterial suspension in a growth medium, such as MHA, it’s crucial to ensure there isn’t an excessive amount of inoculum. This is achieved by pressing the swab on the sides of the bacterial suspension tube before inoculating it onto the MHA plate. Inoculation of the MHA plate with the swab should start from the top, swabbing carefully from side to side down to the bottom of the plate. This step is repeated thrice after each plate rotation (usually 60 degrees) to cover the entire MHA plate with inoculum evenly.

The MIC method delivers the inoculum to each well via panel prongs. These panel prongs containing inoculum must be pressed on all sides and at the centre to ensure the correct volume of bacterial suspension transfers to each well, which is approximately 0.1 ml.

Antimicrobial disks can be added to inoculated MHA plates manually using sterile forceps. Each disk should be placed at equal distances from the others and pressed towards the surface of the agar to prevent disk displacement during incubation.

The inoculated panel can be incubated using the same temperature and time requirements as the MIC method. Additionally, panels should be covered with a plastic seal or in a plastic bag to prevent dehydration since each well contains a minimal bacterial suspension.

Indications: Susceptibility testing for antimicrobials is vital for patients with suspected infections caused by specific pathogens based on disease manifestation and clinical correlation. Antibacterial agents are then employed to determine the sensitivity or resistance of bacteria. This review primarily focuses on susceptibility testing for bacterial pathogens.

Normal and Critical Findings: Reading each set of wells for an antibiotic drug is performed for MIC panels. MIC determination is based on an explicit or slight whiteness in the well. Reporting results of inhibition zones and MIC breakpoints utilize terms like "susceptible" or "resistant" based on the set cut-off range for zone diameter in the nearest whole millimetre and microgram per millilitre, respectively. The Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have developed expert-approved guidelines on breakpoints for reporting results of these methods.

Results

Out of 100 volunteers, there are more male than female volunteers (Figure 2).

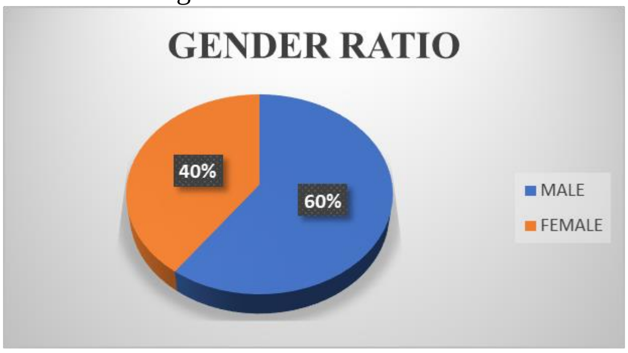
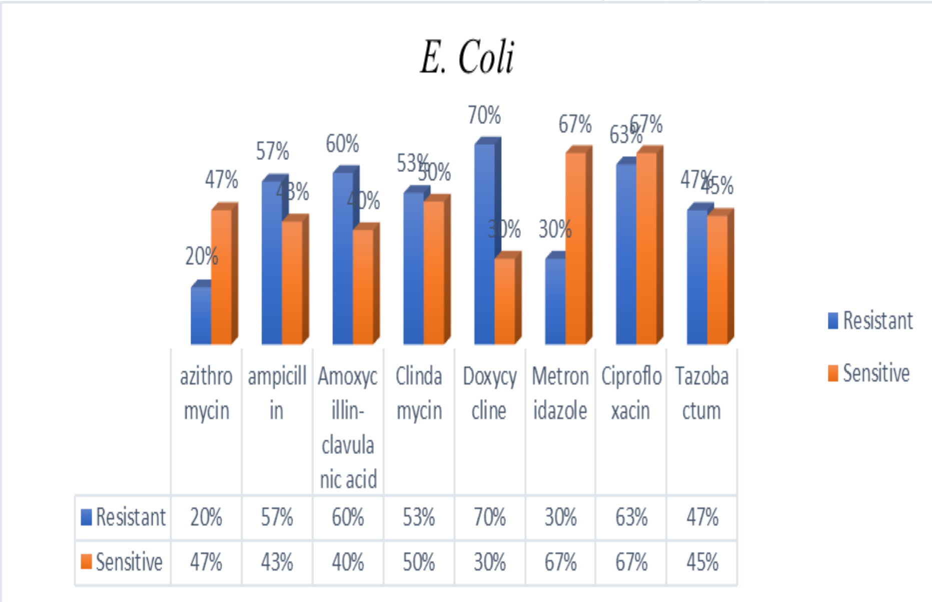


Figure 2: Gender Ratio

The bacterial isolates from the stool samples are *E. coli*, *Clostridium difficile*, *Salmonella typhimurium*, *and Shigella dysenteriae*.

All the antibiotics were tested for their resistance against each bacterium. The bacteria showed a degree of resistance toward each antibiotic.

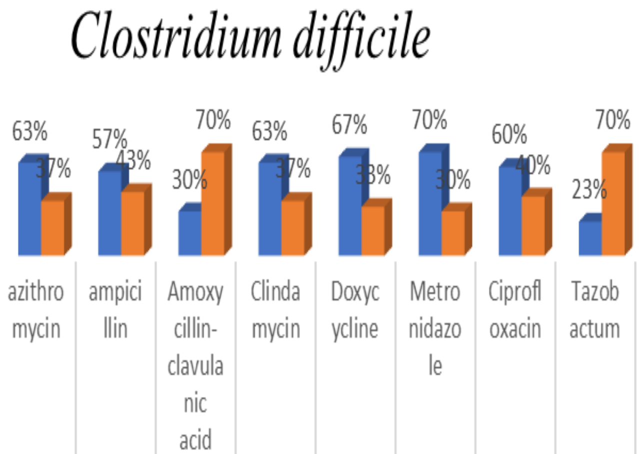
E. coli showed the highest resistance to Doxycycline, followed by Ciprofloxacin and Amoxycillin-clavulanic acid. It was the least resistant to Ampicillin. Regarding sensitivity, *E. coli* is most sensitive to Metronidazole and ciprofloxacin, followed by Tazobactam and Clindamycin (Figure 3).



	azithromycin	ampicillin	Amoxycillin-clavulanic acid	Clindamycin	Doxycycline	Metronidazole	Ciprofloxacin	Tazobactum
Resistant	20%	57%	60%	53%	70%	30%	63%	47%
Sensitive	47%	43%	40%	50%	30%	67%	67%	45%

Figure 3. Resistance and Sensitivity Pattern of *Escherichia coli*

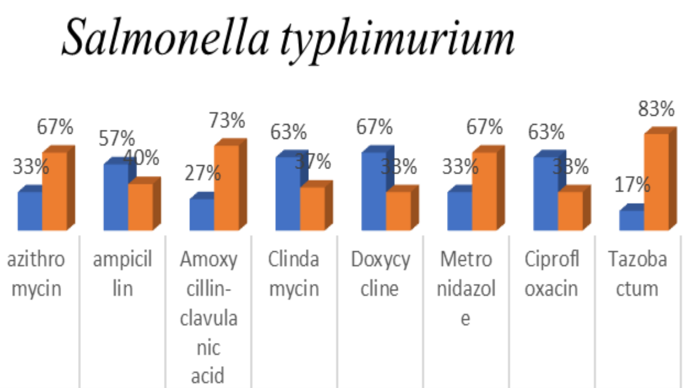
Clostridium difficile showed the highest resistance to Metronidazole, followed by Doxycycline. It was the least resistant to Tazobactam. Regarding sensitivity, *Clostridium difficile* is most sensitive to Amoxycillin-clavulanic acid, Tazobactam, followed by Metronidazole (Figure 4).



	azithromycin	ampicillin	Amoxycillin-clavulanic acid	Clindamycin	Doxycycline	Metronidazole	Ciprofloxacin	Tazobactum
Resistant	63%	57%	30%	63%	67%	70%	60%	23%
Sensitive	37%	43%	70%	37%	33%	30%	40%	70%

Figure 4. Resistance and Sensitivity Pattern of *Clostridium difficile*

Salmonella typhimurium showed the highest resistance to doxycycline, followed by Clindamycin and Ciprofloxacin. It was the least resistant to Tazobactam. Regarding sensitivity, *Salmonella typhimurium* is most sensitive to Tazobactam, followed by amoxicillin acid (Figure 5).



	azithromycin	ampicillin	Amoxycillin-clavulanic acid	Clindamycin	Doxycycline	Metronidazole	Ciprofloxacin	Tazobactum
Resistant	33%	57%	27%	63%	67%	33%	63%	17%
Sensitive	67%	40%	73%	37%	33%	67%	33%	83%

Figure 5. Resistance and Sensitivity Pattern of *Salmonella typhimurium*

Shigella dysenteriae showed the highest resistance to Doxycycline, followed by Amoxycillin-clavulanic acid and Tazobactam. It was the least resistant to Clindamycin. Regarding sensitivity, *Shigella dysenteriae* is most sensitive to Clindamycin followed by Ciprofloxacin (Figure 6).

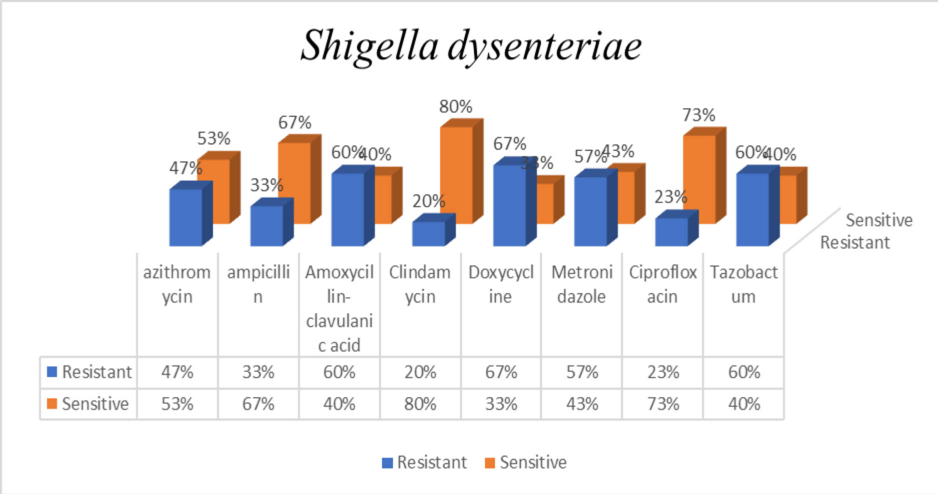


Figure 6. Resistance and Sensitivity Pattern of *Shigella dysenteriae*

Discussion

All the isolates showed a significant amount of resistance to each of the antibiotics. *E. coli* and *Clostridium difficile* are multidrug-resistant organisms. *Salmonella typhimurium* and *Shigella dysenteriae* have still not developed multidrug resistance to antibiotics. *Escherichia coli* (*E. coli*) is a specific subgroup and the most common member of the total coliform group. *Clostridium difficile* is a gram-positive and spore-forming bacterium. It is an obligate anaerobic bacillus that is recognized for its ability to produce toxins and cause diarrhoea. This bacterium also causes colitis, which is inflammation of the colon. This is often associated with antibiotic usage. It exists everywhere around us. This bacterium is communicable in healthcare facilities where healthcare workers come in close contact with patients. Salmonella is a gram-negative, rod-shaped, non-sporing bacterium. *S. Typhimurium* primarily causes typhoid fever in mice, hence the origin of this name. It induces inflammatory diarrhoea in humans. Though it is a less severe pathogen for humans, it is the causative agent of common food-borne enteric infections like gastroenteritis. It is commonly found in foods like eggs, fruits, vegetables, chicken, beef, and processed foods. It is spread through contaminated food items. *Shigellae* are gram-negative, non-spore-forming rod-shaped bacteria. Shigella species cause shigellosis. *Shigella dysenteriae* is a species of the rod-shaped bacterial genus Shigella. *S. Dysenteriae* can invade and replicate in various species of epithelial cells and enterocytes. A lack of water and food quality monitoring, unsanitary cooking conditions, and improper hygiene practices may cause Shigella infections. If hands are unwashed or soiled during food preparation or consumption, it causes contamination.

Conclusion

All species cause some infection in the human body, leading to the consumption of antibiotics. The data shows that few species have started developing multidrug resistance. The diseases spread by these species are communicable. Antibiotic resistance has started growing, and it needs to be controlled. People should maintain proper hygiene while preparing food and while eating. They should be aware of the early symptoms of the infections, reach out to a healthcare professional for the appropriate diagnosis, and get prescribed antibiotics. Antibiotics should be consumed in the prescribed format; people should not stop early as soon as they see signs of progress. This is one of the biggest concerns among the common masses. Healthcare professionals and the government also play an essential part in preventing antibiotic resistance. The primary responsibility of educating the common masses about the significance of consuming antibiotics with proper dosage, side effects, and risks associated with antibiotics lies with the healthcare professionals. They should also make them aware of the dangers of misuse of antibiotics, over-the-counter buying of them, and antibiotic resistance. The importance of good hygiene should also be emphasized to the patients. Over-the-counter sales of antibiotics should be stopped. The government must implement stringent rules across all the pharmaceutical shops in the country so that pharmacists do not sell them without prescriptions.

Together, we need to stop antibiotic resistance before it becomes a pandemic.

Conflict of Interest

At this moment, I declare that there is no conflict of interest.

Acknowledgement

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Ethical Approval

The research protocol, patient information sheet, and informed consent forms were submitted to the Independent Ethics Committee for approval, which was received on 15 September 2023.

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