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Antimicrobial Resistance Trend Pattern in the Population of West Bengal

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Abstract: The world has been struck with a deadly pandemic and is now witnessing severe climate changes. 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 range of broad spectrum as these work on a wide range of disease-causing bacteria (gram-positive and gram-negative). There has been a rise in the accessibility and affordability of antibiotics among the population. The most important reason for this is the over-the-counter buying of antibiotics followed by frequent usage of antibiotics. It is also often observed that patients are not finishing the entire course of antibiotics. Frequent consumption of antibiotics along with not completing the entire course of the antibiotics not only poses a severe threat to the healthy microbiome of the human intestine but also a resistance pattern against the antibiotics. The study aims to understand the antibiotic resistance pattern in the post-COVID scenario on the gut microbiome of the West Bengal population.

Keywords: Antibiotics, antimicrobial resistance, gut microbiome, bacteria,over-the-counter, antibiotic use in India.

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 is causing a fluctuation of temperature and seasonal changes which are ideal causes of viral, and bacterial infections. There is a high burden of various other 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 wide range of disease-causing bacteria (gram-positive and gram-negative)[3]. Common 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 help stop infections caused by bacteria by killing the bacteria or by keeping them from copying themselves or reproducing. The meaning of the word antibiotic is "against life" [5].

Scientists had introduced the first antibiotic, salvarsan, in the early 1900s. Since then, antibiotics have changed medicine and added 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].

The two different types of activity that an antibiotic has are either a narrow or broad spectrum of activity. Narrow-spectrum antibiotics are more specific and are only active against certain 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 abilities to act upon the 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 where oral antibiotics have failed [7].

Antibiotics primarily disrupt essential processes or structures in the bacterial cell. This in turn either kills the bacterium or slows down bacterial growth. An antibiotic is said to be bactericidal or bacteriostatic depending on these effects[6].

A bactericidal antibiotic kills the bacteria while the bacteriostatic antibiotics stop bacterial growth without killing them. The human immune system is then needed to clear the infection[6.] The different classes of bacteria and their mechanisms of action are depicted in Figure 1 [6].

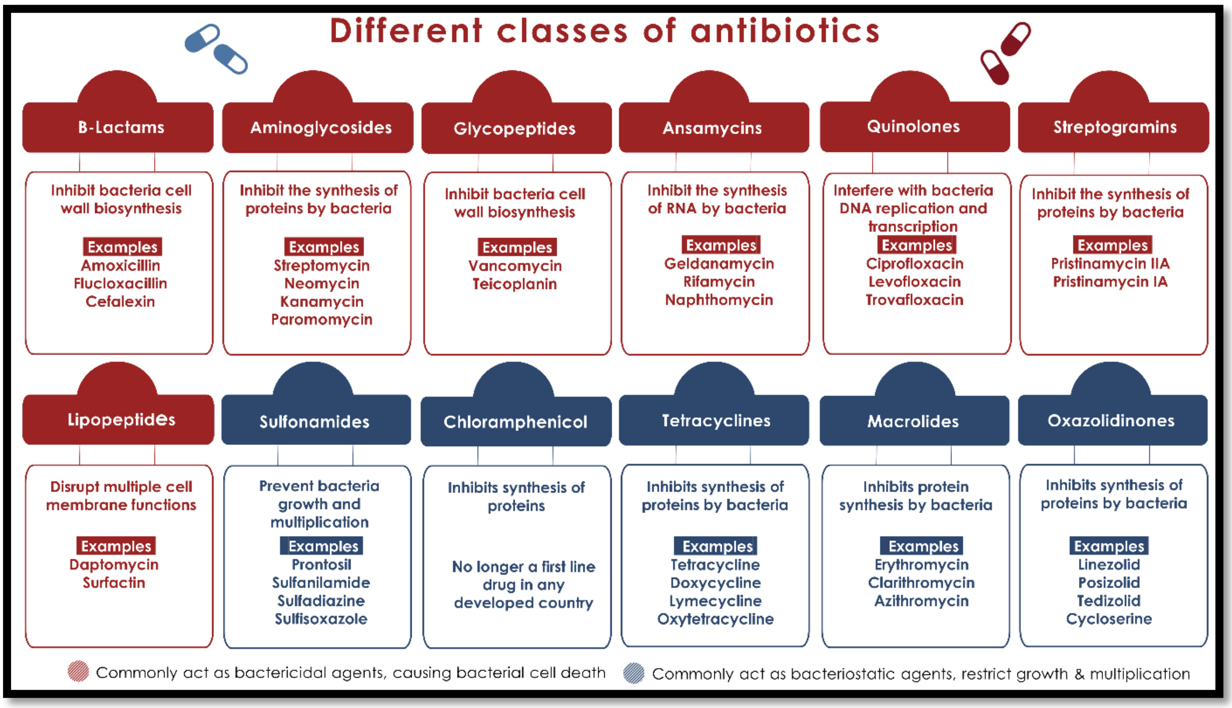

Figure 1: Types and classes of antibiotics

There are several classes of antibiotics with different mechanisms of action and bacterial targets[6]. The three main antibiotic targets in bacteria:

1. The cell wall or membranes that surround 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)

The impact of antibiotic medicine is on the gastrointestinal system thereby disrupting the gut microbiome [8,9]. Early symptoms of an unhealthy gut that are generally found during antibiotic consumption are irritable bowel syndrome and diarrhea[8,10]. Antibiotics can thereby result in several negative consequences 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 diarrhea and recurring C.difficile infections [11]. There is also evidence that early exposure to antibiotics can impact gastrointestinal, immunological, and neurocognitive systems. This is problematic due to the increased use of antibiotics, which suggests a future increase in the prevalence of acute conditions[11].

Eventually, this leads to the development of multidrug-resistant organisms in the gut microbiome thereby beginning the antimicrobial resistance [8,10].

The antimicrobial resistance makes the disease-causing parasites flourish even in the presence of the medication which at one point had impacted their growth [12]. This leads to more complications in recovery and in some extreme cases, the disease-causing pathogen can be resistant to the medications that are presently available [12].

In India, it is very common to buy antibiotics from pharmacy shops without a proper prescription, even though it is illegal. This over-the-counter (OTC) buy results in unregulated sales of antibiotics[1]. The OTC sale of antibiotics is recognized as a pathway for the emergence of antimicrobial resistance (AMR); a serious public health challenge in need of urgent regulatory responses [13].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"[14]. Antimicrobial agents (amas) come under Schedule H and H1[14]. In India, the term OTC is not legally recognized[14].Antimicrobial resistance poses a serious threat in India and worldwide [3,4,8,10]. Globally, India tops the list of countries with the highest antibiotic consumption and AMR. Antimicrobial resistance is a problem causing us to revert to the dark ages when antibiotics were not available [15].

Antibiotic resistance is more severe in developing countries where the burden of infectious diseases is higher and healthcare spending is low[16]. Pan-resistant Gram-negative bacterial infections, which do not have a single sensitive antibiotic are becoming too common in India [16]. Lack of effective regulations, and/or failure to implement the regulations is one of the main reasons behind the inappropriate use of antibiotics. The results conducted from past research across countries revealed that very often, clinicians prescribe antibiotics when they are not required, or they prescribe the wrong antibiotics to patients. Often there are circumstances where pharmacy staff are not fully qualified to sell these medicines, or they sell them without prescriptions.

It is also quite common for patients to not complete the entire course of antibiotics. They tend to stop intake of medicine as soon as 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 commonly used antibiotics now lose their effectiveness in treating the infection 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 the effective treatment of bacterial infections.

Materials and methods

The study was conducted on 30 healthy human volunteers from the city of Kolkata in West Bengal. The age group is 18 years and above. The healthy human volunteers were adequately informed about the study and their consent was duly taken for participation. The volunteers were counseled to not consume any antibiotic 48-72 hours before the collection of their stool sample. The doctor at the clinic checked their vital parameters andhistory 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 McFarlandsuspension
    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 McFarlandturbidity 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 x 10 colony-forming units (CFU) per milliliter. 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 three times after each rotation of the plate (usually 60 degrees) to evenly cover the entire MHA plate with inoculum.

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

The addition of antimicrobial disks on inoculated MHA plates can be done manually using sterile forceps, placing each disk at equal distances from other disks. Each disk should be pressed towards the surface of the agar to prevent disk displacement during incubation.

In the MIC method, the inoculated panel can be incubated using the same temperature and time requirements. Additionally, panels are recommended to be covered with a plastic seal or contained in a plastic bag to prevent dehydration since each well contains a minimal amount of 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: For MIC panels, reading each set of wells for an antibiotic drug is performed. MIC determination is based on either a clear 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 millimeter and microgram per milliliter, 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 30 healthy volunteers,male volunteers are more than female volunteers (Figure 2).

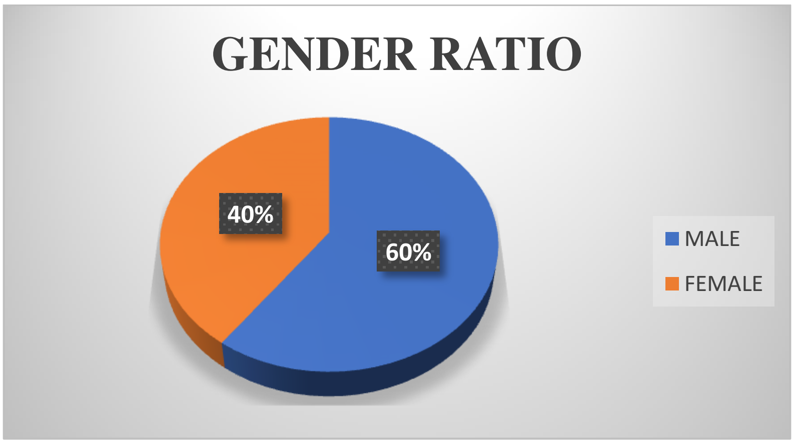

Figure 2

The bacterial isolates from the stool samples areE. 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 towards 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, Ciprofloxacin followed by Tazobactum, and Clindamycin (Figure 3).

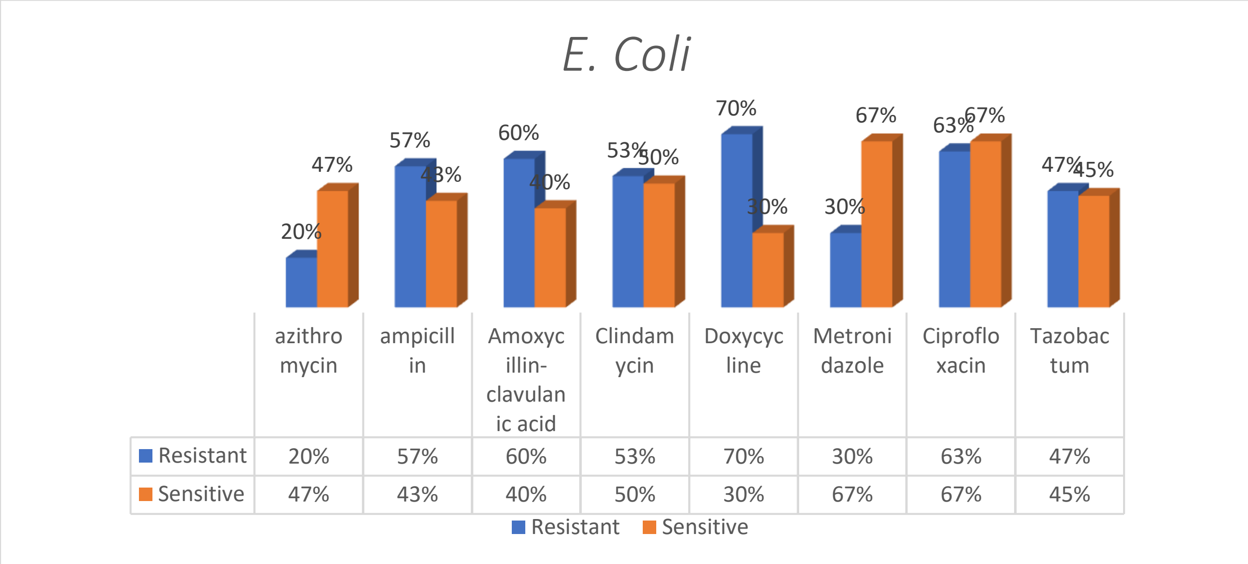

Figure 3

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

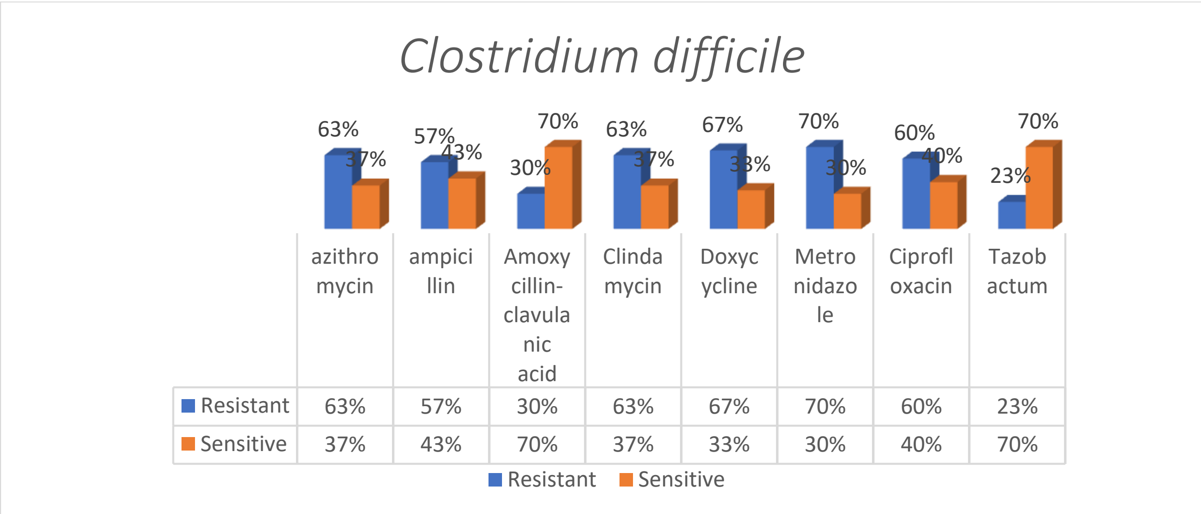

Figure 4

Salmonella typhimurium showed the highest resistance to Doxycycline followed by Clindamycin and Ciprofloxacin. It was the least resistant to Tazobactum. Regarding sensitivity, Salmonella typhimurium is most sensitive to Tazobactumfollowed byamoxycillin-clavulanic acid (Figure 5).

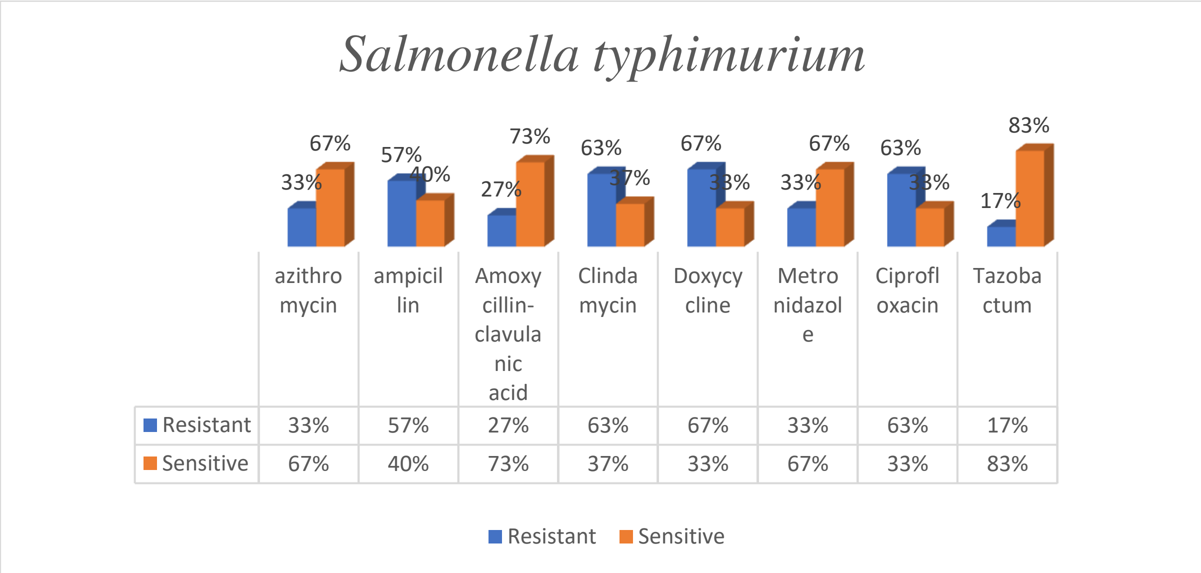

Figure 5

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

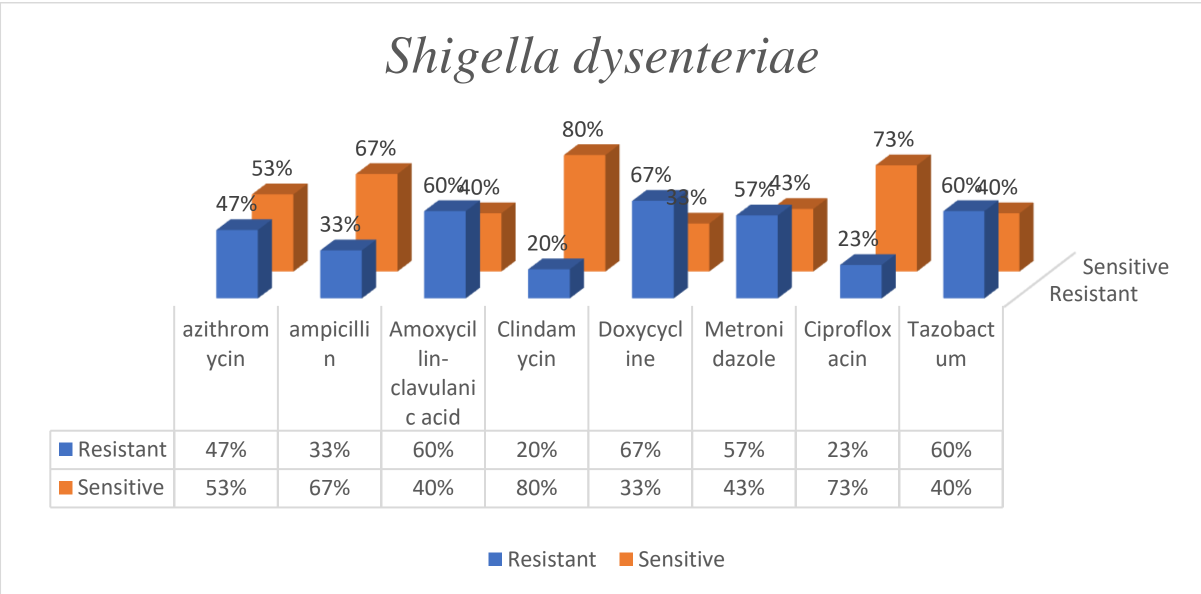

Figure 6

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 diarrhea. 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 healthcareworkers 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 diarrhea in humans. Though it is a less serious pathogen for humansit is the causative agent of common food-borne enteric infections like gastroenteritis. It is commonly found in a variety of foods like eggs, fruits, vegetables, chicken, beef, and even processed foods. It is spread through contaminated food items. Shigellae are gram-negative, non-spore-formingrod-shaped bacteria. Shigella species cause shigellosis. Shigella dysenteriaeis a species of the rod-shaped bacterial genus Shigella.S. Dysenteriae can invade and replicate in various species of epithelial cells and enterocytes.Shigella infections may be contracted by a lack of monitoring of water and food quality, unsanitary cooking conditions, and improper hygiene practices.If hands are unwashed or soiled during food preparation or consumption then causes contamination.

Conclusion

All of the species cause some kind of infection in the human body which leads 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 developing 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 right diagnosis of the infection 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 a role in preventing antibiotic resistance. The primary responsibility of educating the common masses about the importance 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. They should also emphasize the importance of good hygiene to the patients. Over-the-counter sales of antibiotics that need to be stopped. The government should implement strict 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: I hereby declare that there is no conflict of interest.

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References

1. Das, S., Agarwal, S., Bose, A., Das, D., (2024). Intestinal microbiome and antimicrobial resistivity: A study in recent times. Texila Internation Journal of Academic Research 2014.11.02. Art011.
2. Barron, M.,(2022). The Gut Resistome and the Spread of Antimicrobial Resistance. American Society of Microbiology, June 2022.
3. Singh, S., Verma, N., Taneja, N., (2019). The human gut resistome: Current concepts & prospects. Indian Journal of Medical Research, 150(4): 345–358.
4. Ezmed., Antibiotic Chart: Drug Names, Coverage and Mechanism of Action, Jan 2024.
5. Powell, A., Sheikh, Z., (2024) Antibiotics: Everything you should know.Webmdarticle.
6. React, SIDA, Uppsala University, (2024). How do antibiotics work?
7. Pascaul, C., Vergara, A., Vila, J.,(2018). Intestinal microbiota and antibiotic resistance: Perspectives and solutions. Human Microbial Journal, vol. 9, 11-15.
8. Yang, L., Bajinka, O., Jarju, P., Tan, Y., Taal, A., Ozdemir, G., (2021). The varying effects of antibiotics on gut microbiota. AMB Expr, 11:116.
9. Matzaras, R.,Nickopoulou, A., Protonotariou, E., Christaki, E.,(2022). Gut Microbiota Modulation and Prevention of Dysbiosis as an Alternative Approach to Antimicrobial Resistance: A Narrative Review. Yale J Biol Med, 95(4): 479–494.
10. Aliouche, H., (2022). The Effect of Antibiotics on Gut Microbiome. News Medical Life Sciences.
11. WHO Report (2020) Antibiotic Resistance.
12. Porter, G., Kotwani, A., Bhullar, A., Joshi, Jyoti., (2021). Over-the-counter sales of antibiotics for human use in India: The challenges and opportunities for regulation. Medical Law International, Vol. 21(2) 147–173.
13. Kotwani, A., Joshi, J., Lamkang, A., (2021). Over-the-counter sale of antibiotics in India: A qualitative study of providers' perspective of across two states. 10(9): 1123.
14. Ghafur, A., (2010). An obituary- On the death of antibiotics. Journal of the Association of Physicians of India. 58(3): 143-4.
15. Salunkhe, D., Pandit, A., Dawane, S., Sarda, D., (2013). More CS Study of over-the-counter sale of antimicrobials in pharmacy outlets in Pune, India:A cross-sectional study. International J Pharma Bio Sci. 4:616.