STUDY OF ANTIMICROBIAL RESISTANCE IN A TERTIARY HEALTHCARE FACILITY

A Thesis Submitted

in Partial Fulfilment of the Requirements

for the Degree of

Bachelor of Technology

By

Krishnanu Choudhuri (00419020)

Satarupa Deb Sinha (00419028)

Under the guidance of

Dr. Sucheta Das Maji

to the

Department of Biotechnology

Haldia Institute of Technology

June - 2023

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LIST OF ABBREVIATIONS

AMR-Antimicrobial Resistance

WHO- World Health Organization

HAI- Healthcare-Associated Infections

MDR- Multi drug resistance

ESBL- Extended-Spectrum β-Lactamases

MRSA- Methicillin-resistant *Staphylococcus aureus*

VRE - vancomycin-resistant *Enterococcus faecium*

TSB- Tryptic Soy Broth

LB- Luria Bertani (media)

LEGEND OF FIGURES AND PLATES

LEGENDS OF TABLES AND FLOW-CHART

SYNOPSIS

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ABSTRACT

In the case of severe hospital acquired infections (HAI), antimicrobial resistance (AMR) poses a substantial threat to human life. This research article highlights the increasing problem of antibiotic resistance among different species of organisms, and the lack of awareness towards monitoring the same. The observational study in accordance with the results obtained aims to gather data and give inference on the culture and sensitivity of organisms isolated from various wards of a tertiary healthcare facility in Haldia, West Bengal and to assess the possible factors favouring the development of AMR. This data will help authorities to maintain standard healthcare guidelines towards the patients through awareness and for the proper and effective use of antibiotics. Results associated with sensitivity of organisms towards the antibiotics were collected from performing experiments in the Microbiology laboratory facility of Haldia Institute of Technology. The study collected data on 12 samples which were incubated and observed before isolating. The study found considerably significant resistance to some commonly prescribed first-line antimicrobials such as Amoxicillin, Cloxacillin, Gentamicin, Ceftazidime, Levofloxacin, Azithromycin, Amikacin and Ceftriaxone. Out of 12 samples, one showed significant resistance towards more than one antibiotic. Therefore, the data gathered suggests that it is crucial to regularly monitor antimicrobial resistance and rotate the antibiotic uses in order to prevent the further development of resistance. The study also emphasises the significance of educating and increasing public knowledge of the negative effects of improper and excessive usage of antibiotics.

Keywords: Antibiotics, antimicrobial resistance, bacteria, antibiotic sensitivity test, tertiary healthcare facility, awareness

CONTENTS

INTRODUCTION

Antimicrobial resistance (AMR) refers to the ability of microorganisms, particularly bacteria, to develop resistance against the drugs designed to kill or inhibit their growth. This resistance occurs when microorganisms produce mutant variants that fail to respond to medicines with time. This phenomenon poses a significant threat to public health globally and has the potential to undermine the effectiveness of many medical interventions [1]. The World Health Organisation (WHO) has recognised AMR as one of the top ten global health threats due to its impact on the effective treatment of infectious diseases [2]. The first antibiotic, penicillin, was discovered by Alexander Fleming in 1928. Soon after its introduction into clinical practice, reports of bacterial resistance to penicillin began to emerge in the 1940s. This necessitated the development of second-generation antibiotics in the 1960s, which were designed to combat the newly resistant strains of bacteria. However, the cycle continued, and by 1981, resistance to these newer antibiotics was also observed. Since the discovery of penicillin, numerous antibiotics have been developed, but the emergence of resistance has been a recurring problem. The misuse and overuse of antibiotics in human medicine, animal agriculture, and even in the environment have contributed to the rapid development and spread of resistant strains of bacteria. One of the most concerning aspects of AMR is the emergence of multidrug-resistant (MDR) organisms. These are bacteria that have acquired resistance mechanisms against multiple antibiotics, making them difficult to treat. MDR bacteria pose a serious threat to healthcare settings, as they can cause infections that are challenging to manage and control. This can result in prolonged hospital stays, increased healthcare costs, and higher mortality rates.

Furthermore, this study can contribute to the broader understanding of antimicrobial resistance (AMR) and its mechanisms. By investigating the genetic and biochemical changes associated with antibiotic resistance, the study can contribute to the knowledge base on AMR and potentially aid in the development of new strategies for combating antibiotic resistance. The study is important because it provides valuable insights into the presence and characteristics of antibiotic-resistant microorganisms in the hospital environment. By monitoring the air microflora, the study can identify potential sources of antibiotic resistance and inform infection control practices within the hospital.

Causes of AMR

Antimicrobial resistance (AMR) is a complex issue influenced by multiple intrinsic and extrinsic factors. Extensive research on the causes of AMR has identified several key

contributors. Firstly, the overuse and misuse of antibiotics in human and animal health sectors play a significant role. Inappropriate prescribing practices, self-medication, incomplete courses of treatment, and the widespread use of antibiotics in livestock production contribute to the selection and proliferation of resistant strains. Secondly, the inadequate access to clean water, sanitation, and hygiene facilities, particularly in low- and middle-income countries, promotes the transmission and persistence of resistant bacteria. Thirdly, the global spread of AMR is facilitated by international travel and trade, enabling the movement of resistant organisms and resistance genes across borders. Additionally, the widespread use of antibiotics in agriculture, particularly as growth promoters, contributes to the selection and dissemination of resistance in the environment. Moreover, poor infection prevention and control practices in healthcare settings can lead to healthcare-associated infections caused by resistant bacteria [22]. Finally, the limited development of new antibiotics and the slow pace of innovation in the field exacerbate the problem by reducing treatment options for infections caused by resistant pathogens. Addressing these causes requires a multifaceted approach involving effective surveillance, rational use of antibiotics, improved hygiene practices, responsible use of antibiotics in agriculture, and investment in research and development of new antimicrobial agents.

Mechanism of Antimicrobial Resistance

Antimicrobial resistance mechanisms can be classified into four main categories. The first mechanism is antibiotic inactivation, which occurs when bacteria possess plasmids, which are extrachromosomal genetic material that carry genes responsible for resistance against specific antibiotics. These genes encode enzymes that can modify or degrade the antibiotic molecules, rendering them ineffective [4]. The second mechanism is target modification, where bacteria develop resistance by altering the target sites of antimicrobial agents. Normally, these agents bind to specific sites in bacterial cells and disrupt their normal functions. However, through genetic changes, bacteria can modify these target sites, making them less susceptible to the action of antibiotics. The third mechanism is the efflux mechanism of resistance. Bacterial genomes inherently possess efflux pump proteins encoded by specific genes, which play a role in maintaining cellular functions. These efflux pumps can pump out antibiotics from the bacterial cells, preventing them from reaching effective concentrations and thereby conferring resistance. The fourth mechanism is plasmidic efflux, which involves the acquisition of resistance genes from other resistant organisms. Bacterial species can exchange genetic material through processes such as transformation (uptake of free DNA from the environment), conjugation (transfer of DNA

through direct cell-to-cell contact), and transduction (transfer of DNA mediated by bacteriophages). This horizontal transfer of genetic material allows bacteria to acquire new resistance genes, leading to the development of resistance. These mechanisms collectively contribute to the emergence and spread of antimicrobial resistance in bacteria. Understanding these mechanisms is crucial for developing effective strategies to combat resistance, including the development of new antibiotics, the implementation of antimicrobial stewardship programs to promote appropriate antibiotic use, and infection control measures to prevent the spread of resistant bacteria.

Current Scenario of AMR

AMR is a global health crisis, causing a significant number of deaths each year and posing a substantial economic burden [3,6]. The World Health Organization and other stakeholders have recognized the urgent need to address AMR [5]. In India, the situation is particularly complex, as AMR affects not only humans but also livestock and the environment. India has been referred to as the AMR capital of the world due to various factors such as a large and diverse population, the emergence of multi-drug resistant genes, and inadequate waste disposal management [7]. In human healthcare settings, high rates of antibiotic resistance have been observed among Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* [17,20]. These bacteria have shown resistance to fluoroquinolones and third-generation cephalosporins, which are commonly used antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) have also been identified, indicating the presence of antibiotic-resistant Gram-positive organisms. Additionally, *Salmonella Typhi* and *Shigella* species have exhibited resistance to commonly used antibiotics like ciprofloxacin, ceftriaxone, and co-trimoxazole. The AMR problem extends beyond human health, with significant resistance observed in livestock as well [18]. In milk samples, a high percentage of Gram-negative bacilli were found to be extended-spectrum β-lactamases (ESBL) producers, indicating resistance to important antibiotics. Furthermore, resistance to oxytetracycline was observed in a considerable proportion of the isolated bacteria. Similar resistance patterns have been reported in poultry, with studies documenting the presence of ESBL-producing *Enterobacteriaceae* and high rates of *Salmonella* species resistant to multiple antibiotics. The contamination of major water bodies in India, such as the Ganges, Yamuna, and Kaveri, by domestic waste, hospital effluents, and pharmaceutical releases further exacerbates the AMR crisis. E. coli isolated from these water bodies have demonstrated resistance against third-generation cephalosporins in varying percentages.

The current scenario of AMR in India is alarming and calls for immediate attention [10]. Efforts are needed to strengthen antimicrobial stewardship programs, enhance infection control measures, promote responsible use of antibiotics in both humans and animals, and improve waste management practices. Additionally, research and development of new diagnostic tools and therapeutics are essential to combat the growing threat of AMR. Addressing AMR requires a comprehensive and coordinated approach involving healthcare professionals, policymakers, researchers, and the public to preserve the effectiveness of antibiotics and ensure the sustainability of healthcare systems.

OBJECTIVES

The objective of the study is to monitor the air microflora in different areas within a hospital located in Haldia. The purpose of this monitoring is to assess the microbial diversity and abundance present in the hospital environment. The aim of this testing is to identify any microorganisms that have developed resistance to commonly used antibiotics. In addition to antibiotic susceptibility testing, the study also aims to perform biochemical characterization of the antibiotic-resistant mutants.

LITERATURE REVIEW

Origin

The origin of antimicrobial resistance (AMR) is a multifaceted process influenced by various genetic, evolutionary, and ecological factors. Understanding the origin of AMR is crucial for developing effective strategies to combat its spread and impact. Genetic mutations are a natural occurrence within microorganisms and can give rise to resistance against antimicrobial agents. These mutations can arise spontaneously through errors during DNA replication or as a result of exposure to selective pressures, such as the presence of antibiotics. Microorganisms with mutations that provide a survival advantage in the presence of antibiotics can outcompete susceptible strains, leading to the emergence of resistant populations. Horizontal gene transfer is another important mechanism driving the spread of resistance genes among bacteria [4]. Mobile genetic elements, such as plasmids and transposons, facilitate the transfer of resistance genes between different bacteria, even across species boundaries. This transfer can occur through processes like conjugation, where genetic material is directly transferred from one bacterium to another, or through transduction, where genetic material is carried by bacteriophages (viruses that infect

bacteria). Horizontal gene transfer allows bacteria to rapidly acquire new resistance mechanisms, leading to the emergence of multidrug-resistant strains. Environmental reservoirs, such as soil and water, play a significant role in the development and dissemination of antimicrobial resistance. These reservoirs serve as hotspots for the exchange of genetic material between bacteria. Agricultural practices, including the use of antibiotics in livestock and aquaculture, can contribute to the dissemination of resistance genes through the environment. Antibiotics used in human healthcare also find their way into the environment through wastewater and pharmaceutical waste, further contributing to the selective pressure for resistance. The widespread use and misuse of antibiotics in human healthcare, veterinary medicine, and agriculture significantly contribute to the emergence and spread of AMR. Inappropriate and excessive use of antibiotics, including unnecessary prescriptions, failure to complete full courses of treatment, and over-the-counter availability, create selective pressures that favour the survival and proliferation of resistant bacteria. This selective pressure is further exacerbated by self-medication practices and the availability of antibiotics without a prescription in some regions. The overuse of antibiotics in livestock farming for growth promotion and disease prevention also contributes to the development of resistant bacteria that can spread to humans through the food chain.

The interplay of these factors leads to the emergence and spread of AMR, posing a serious global health challenge [21]. Addressing AMR requires comprehensive strategies that focus on reducing the inappropriate use of antibiotics, promoting responsible antibiotic stewardship, improving infection prevention and control measures, and investing in the development of new antimicrobial agents. A One Health approach, which recognizes the interconnectedness of human, animal, and environmental health, is crucial for effectively tackling the complex issue of antimicrobial resistance and safeguarding the effectiveness of antibiotics for future generations.

Antibiotic discovery and resistance timeline

The timeline of antibiotic discovery and resistance reflects the ongoing battle between the development of new antibiotics and the evolution of resistance mechanisms in bacteria. The story begins with the groundbreaking discovery of penicillin by Alexander Fleming in 1928, marking the dawn of the antibiotic era [19]. This discovery revolutionised medicine and paved the way for the development of other antibiotics throughout the mid-20th century. In the 1940s and 1950s, as penicillin became widely used, the first signs of

antibiotic resistance emerged. Staphylococcus aureus, a common bacterium responsible for various infections, including skin and respiratory tract infections, developed resistance to penicillin. This led to the development of new antibiotics, such as methicillin and other β-lactam antibiotics, to combat these resistant strains [12]. However, bacteria continued to evolve and acquire new resistance mechanisms. By the 1960s and 1970s, the emergence of multidrug-resistant organisms became a growing concern. Bacteria were found to develop resistance to multiple antibiotics, making treatment options increasingly limited [17]. The production of β-lactamases, enzymes that can inactivate β-lactam antibiotics, became a prominent resistance mechanism. Efforts were made to develop new antibiotics, including the introduction of carbapenems and fluoroquinolones in the 1980s. However, resistance to these new agents also emerged, highlighting the adaptive nature of bacteria. The 1990s and early 2000s witnessed the alarming spread of antibiotic-resistant pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) gained prominence as major public health threats [9]. These resistant strains posed significant challenges in healthcare settings, leading to increased morbidity, mortality, and healthcare costs. The rise of community-associated MRSA infections further heightened concerns about antibiotic resistance in the general population. In recent years, there has been a growing recognition of the urgent need to address antibiotic resistance as a global health crisis. The World Health Organization (WHO) and other international organisations have emphasised the importance of responsible antibiotic use, infection prevention and control, and the development of new antimicrobial agents [23]. Efforts are underway to promote antimicrobial stewardship programs, educate healthcare providers and the public about the appropriate use of antibiotics, and invest in research and development for new antibiotics and alternative treatment options. The timeline of antibiotic discovery and resistance underscores the ongoing challenge of staying ahead of bacterial evolution. It highlights the need for a multifaceted approach that combines the prudent use of antibiotics, robust infection control measures, and innovative strategies to combat the emergence and spread of antibiotic-resistant bacteria. Addressing antibiotic resistance requires global collaboration, policy interventions, and sustained efforts to preserve the effectiveness of antibiotics for future generations.

Consequences

Antibiotic resistance has far-reaching consequences, both at the individual health level and for public health as a whole. At the individual level, the consequences of antibiotic

resistance can be severe. When infections are resistant to antibiotics, individuals may experience prolonged illness, increased severity of symptoms, and a higher risk of complications [11]. Treatment failure becomes more common, leading to the need for alternative or stronger antibiotics, which may have more side effects and be less effective. This can result in longer hospital stays, more frequent doctor visits, and increased healthcare costs for individuals and their families. In some cases, antibiotic-resistant infections may even lead to disability or death. Beyond the individual level, antibiotic resistance has significant public health implications [8]. Resistant bacteria can spread within communities, healthcare facilities, and even across countries, facilitated by global travel and trade. This makes it more challenging to control the transmission of infectious diseases and increases the risk of outbreaks. In healthcare settings, the presence of antibiotic-resistant bacteria can lead to the spread of infections among vulnerable populations, such as elderly individuals or those with weakened immune systems. It also puts healthcare workers at risk of contracting and transmitting these infections. The burden on healthcare systems increases as more resources are needed to manage antibiotic-resistant infections and prevent their spread. Antibiotic resistance also undermines the effectiveness of preventive measures. For example, in surgical procedures, prophylactic antibiotics are often administered to prevent postoperative infections. However, when bacteria are resistant to these antibiotics, the effectiveness of this preventive measure is diminished, leading to higher rates of surgical site infections. Similarly, in vulnerable populations such as newborns or individuals undergoing chemotherapy, antibiotics are often used as a preventive measure to protect against infections. However, antibiotic resistance reduces the effectiveness of these preventive strategies, putting these individuals at greater risk. In the long term, antibiotic resistance poses a significant threat to modern medicine. The loss of effective antibiotics hinders the ability to treat common bacterial infections, making once easily treatable conditions more challenging to manage. This impacts various medical interventions, including surgeries, as the risk of postoperative infections increases. It also affects treatments for diseases like cancer, where chemotherapy can weaken the immune system and make individuals more susceptible to infections. Organ transplants, which rely on effective antibiotics to prevent and treat infections, may also become more risky and less successful [21]. To address the consequences of antibiotic resistance, it is essential to implement comprehensive strategies. This includes promoting appropriate and responsible use of antibiotics in human and animal health, improving infection prevention and control measures in healthcare settings, investing in research and development for new antibiotics

and alternative treatments, and raising awareness among healthcare professionals and the general public about the importance of prudent antibiotic use. By taking action to combat antibiotic resistance, we can protect individual health, preserve the effectiveness of antibiotics, and ensure the continued success of medical interventions and public health efforts [16].

Diseases associated with AMR

Antimicrobial resistance (AMR) has given rise to a multitude of infections and diseases that present significant challenges to global health. In the realm of bacterial infections, AMR has had a profound impact on healthcare-associated infections. Resistant strains have been observed in bloodstream infections, making them more difficult to treat and potentially leading to severe complications. Urinary tract infections, which are commonly encountered, have also been affected by AMR, with resistant bacteria posing challenges in finding effective treatment options. Surgical site infections have become increasingly problematic due to the emergence of resistant strains, potentially leading to postoperative complications and increased healthcare costs. Notable examples of bacteria that have developed resistance to multiple antibiotics include methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. MRSA, in particular, has become a significant concern in both healthcare and community settings. It causes a range of infections, from skin and soft tissue infections to severe bloodstream infections, and is associated with increased morbidity and mortality. ESBL-producing Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae*, have become increasingly prevalent and pose a challenge in treating infections, particularly urinary tract and bloodstream infections [13]. Respiratory tract infections, such as pneumonia and tuberculosis, have also been impacted by AMR. The emergence of resistant strains makes the management of these infections more complex, requiring alternative treatment options and increased vigilance in monitoring treatment outcomes. Sexually transmitted infections, such as gonorrhoea, have seen a rise in antimicrobial resistance, making treatment more challenging and potentially leading to complications and increased transmission rates [24]. In the context of viral infections, antiviral resistance has been observed in diseases such as HIV/AIDS and influenza. The development of resistance to antiviral medications limits treatment options and increases the risk of treatment failure, impacting both individual patients and public health efforts to control these infections. Furthermore, the emergence of multidrug-resistant fungi,

including *Candida* and *Aspergillus* species, has complicated the management of fungal infections, particularly in immunocompromised individuals. These resistant fungal strains can cause severe infections, such as invasive candidiasis and invasive aspergillosis, and are associated with high morbidity and mortality rates. It is crucial to promote judicious and appropriate use of antimicrobials, both in healthcare settings and in agriculture, to preserve the effectiveness of existing treatments and prevent the further emergence and spread of AMR. Additionally, efforts to strengthen healthcare systems, improve surveillance and monitoring of resistance patterns, and promote research and innovation in antimicrobial development are essential to address the challenges posed by AMR and safeguard public health [14,15].

MATERIALS & METHODS

Materials Required

- Culture plates
- Eppendorfs
- Culture media (TSB and/or LB)
- Pipette-man
- Pipette tips (Autoclaved)
- Alcohol
- Tissue
- Lamp
- Matches
- Spreader
- Inoculation loop
- Samples
- KBO09 HiCarbohydrate™ Kit

Media Used

Isolation of microorganisms were performed on Trypticase Soy Agar plates and they were preserved on LB (Luria-Bertani Broth) slants. To prepare Trypticase Soy Broth (TSB) media, 30gm TSB powder was added to 1000ml distilled water. For plating, 2% agar was added to solidify the media. The media was then autoclaved at 121ºC for 15 mins at 15 psi

pressure. All the glassware and apparatuses used were properly cleaned and sterilised to reduce the chances of contamination.

Exposure of the plates and isolation of bacteria

Autoclaved TSB-agar media was evenly distributed into sterile petri plates, and sealed immediately after solidifying to avoid contamination. The plates were then taken to a tertiary care hospital and exposed for 30 mins at four different zones within the hospital area (ICU, OPD, GENERAL WARD, RECEPTION). The plates were again sealed after exposure and brought back in icebox to the laboratory for incubation at 37ºC for 24 hours.

Morphological Characterization

For characterization of bacterial isolates, standard identification protocols were performed. After 24 hours of incubation around 12 different varieties of colonies were found to appear on the petri plate. Based on their different colony morphology, these colonies were then separated to fresh petri plates and allowed to multiply.

Biochemical characterization of the isolates

For identification of the most resistant isolates some biochemical assays were performed following standard protocol (Gram staining, Catalase test, Oxidase test, Arginine hydrolysis test and IMViC test).

Study of Antibiotic Sensitivity

Overnight cultures of selected isolates were spread over TSB plates and sterile antibiotic discs were carefully placed over them (Kirby-Bauer Sensitivity Test). The plates were kept for incubation at 37ºC for 24hrs to observe the antibiotic sensitivity. Antibiotics namely Amoxicillin, Cloxacillin, Gentamicin, Ceftazidime, Levofloxacin, Azithromycin, Amikacin and Ceftriaxone were used against the bacterial isolates to check their resistance ability.

List 1: Antibiotics used along with their respective concentrations:

Gentamicin - 10 mcg/disc
Amoxicillin - 10 mcg/disc
Ceftazidime - 30 mcg/disc

Azithromycin - 15 mcg/disc

Cloxacillin - 1 mcg/disc

Levofloxacin - 5 mcg/disc

Ceftriaxone - 30 mcg/disc

Amikacin - 30 mcg/disc

RESULT AND DISCUSSION

Study of air microflora in the hospital environment

After 24 hours of incubation, around 12 varieties of colonies appeared on the exposed plates (Figure 1). They were named as W1, W2, W3, W4, W6, I1, I2, I3, R1, R2, R3 and O1. They are preserved in glycerol stock for future use.

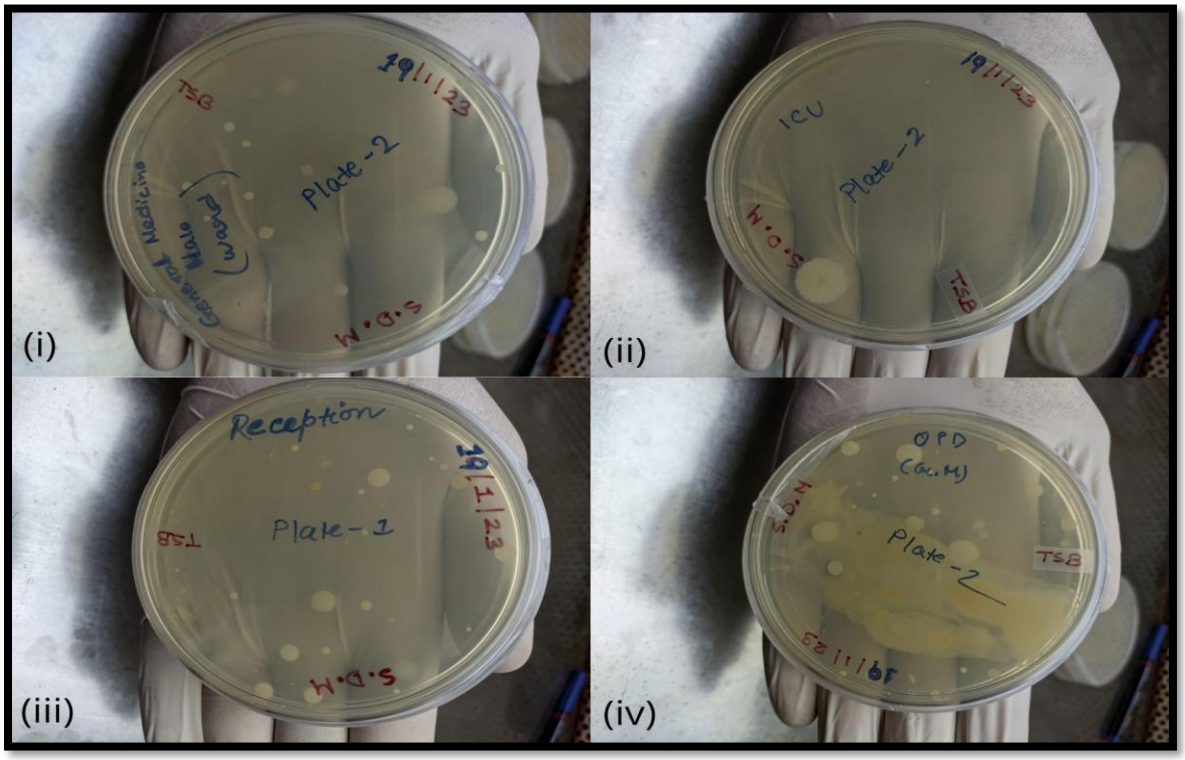

Figure 1: Images of the four plates showing varieties of bacterial colony after 24 hours of incubation that were exposed at different hospital areas namely (i) General Ward (W) (ii) ICU (I) (iii) Reception (R) (iv) OPD (O)

Antimicrobial resistance assay

Table 1 shows the result of antimicrobial assay done against 12 isolates. Figure 2 shows some of the experimental results.

Table 1: Antibiotic Resistance Test Observation (Kirby - Bauer Test) Set-1

Diameter of Zones around antibiotic discs (in cm)								
Bacterial Strains	**Amikacin**	**Azithromycin**	**Amoxicillin**	**Levofloxacin**	**Ceftazidime**	**Cloxacillin**	**Gentamicin**	**Ceftriaxone**
W1	2	2.1	-	1.4	2.3	1.7	1.5	2.2
W2	1	2	1.5	2.5	2	1.5	-	1.8
W3	1.4	-	-	3.2	3.9	3	1.8	3.3
W4	3.7	-	2.6	3.5	3.2	3.5	1.5	3.3
W6	1.9	1.2	2	2.7	2.2	2.7	1.7	1.9
O1	2.1	1.5	-	-	3.7	2.4	2.1	3.8

Table 2: Antibiotic Resistance Test Observation Set-2

Diameter of Zones around antibiotic discs (in cm)								
Bacterial Strains	**Amikacin**	**Azithromycin**	**Amoxicillin**	**Levofloxacin**	**Ceftazidime**	**Cloxacillin**	**Gentamicin**	**Ceftriaxone**
R1	1.8	-	-	3.3	4.5	2.5	1	4
R2	2.1	1.1	-	3	4.4	2	1	4.2
R3	1.9	-	2	3.2	4.3	2.9	1.8	4
I1	2.3	-	-	2	3.6	2.3	1.5	3.1
I2	1.5	1.5	-	-	-	-	2.2	1.9
I3	-	2	-	-	3	2.1	2.5	3.7

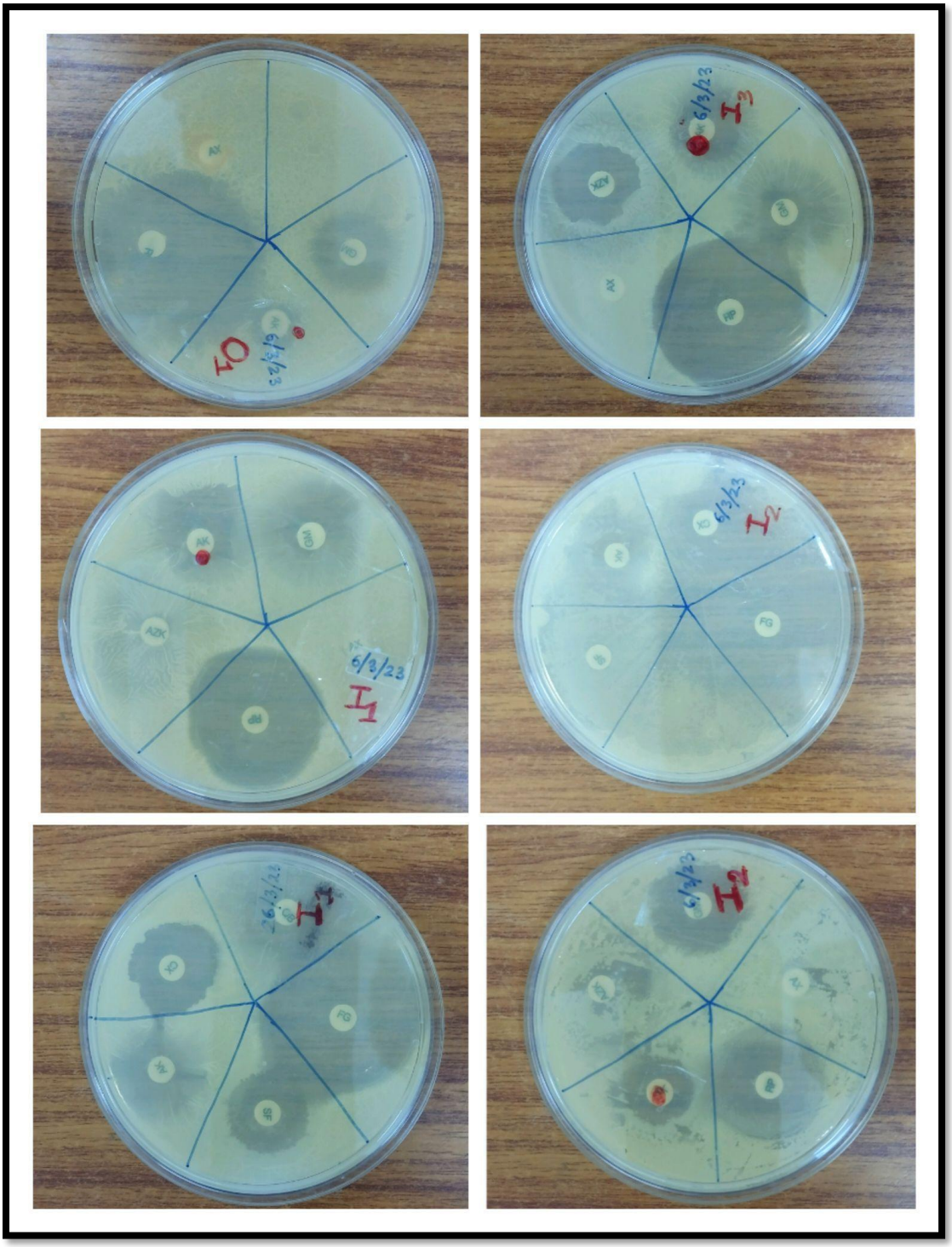

Figure 2: Antibiotic Sensitivity Assay Plates

From the antibiotic sensitivity assay it was found that isolate I2 was most resistant to the antibiotics tested. So, this strain was taken for further experiments. Bacterial strains showed the most resistance (no zones) against Azithromycin, Amoxicillin and Levofloxacin.

Morphological Assay

For identification of the bacterial isolates some morphological assays were performed following standard protocol. Each of the bacterial isolates were observed under a compound microscope and their shapes were identified. The following table shows the details of the isolates.

Table 3: Morphological Assay Results

Strain	**Shape**
W1	mixed(rod+cocci)
W2	rod
W3	cocci
W4	mixed
W6	cocci
I1	mixed
I2	cocci
I3	cocci
O1	rod
R1	spirillum
R2	rod
R3	mixed

Table 4: Gram staining table

Bacterial Strains	**Gram Staining Result**
W1	+
W2	+
W3	+
W4	+
W6	+
I1	+
I2	+
I3	+
O1	+
R1	+
R2	+
R3	+

From the above observations it can be concluded that the strain I2 showed the most resistance against multiple antibiotics. Hence some biochemical assays and other tests were performed for the identification of the strain.

Table 5: Biochemical Assay Results

TESTS/ISOLATE	**I2**
Catalase test	+
Oxidase test	+
Arginine hydrolysis	+
Indole test	+
Methyl Red test	+
Voges-Praueskauer test	-
Citrate Utilisation test	+
Nitrate Reduction test	-

Table 6: Carbohydrate utilisation test

No.	**Test**	**Bacterial Strain(I2)**
1	Lactose	+
2	Xylose	+
3	Maltose	+
4	Fructose	+
5	Dextrose	+
6	Galactose	+

No.	Test	Bacterial Strain(I2)
7	Raffinose	+
8	Trehalose	+
9	Melibiose	+
10	Sucrose	+
11	L-Arabinose	+
12	Mannose	+
13	Insulin	+
14	Sodium Gluconate	+/-
15	Glycerol	+
16	Salicin	+
17	Dulcitol	+/-
18	Inositol	+
19	Sorbitol	+
20	Mannitol	+
21	Adonitol	+/-

No.	Test	Bacterial Strain(I2)
22	Arabitol	+/-
23	Erythritol	+/-
24	α-Methyl-D-glucoside	+/-
25	Rhamnose	+/-
26	Cellobiose	-
27	Melezitose	+/-
28	α-Methyl-D-mannoside	+/-
29	Xylitol	+/-
30	ONPG	+/-
31	Esculin hydrolysis	+
32	D-Arabinose	+
33	Citrate utilisation	-
34	Malonate utilisation	-
35	Sorbose	-

Figure 3: Images showing initial and final results of carbohydrate utilisation by the most resistant bacterial strain(I2). KBO09 HiCarbohydrate^{TM} Kit was used to perform the test.

Discussion

- From the above tests and assays it can be predicted that the resistant isolate (I2) belongs to *Bacillus* sp.
- Specifically, the bacterial strain I2 showed high levels of resistance (no zones) to Amoxicillin, Levofloxacin, Ceftazidime, Cloxacillin and Azithromycin (weak zone).
- The commonly used antibiotics including Amoxicillin and Azithromycin, were primary drugs against which resistance was exhibited by more than 8-9 organisms.

CONCLUSION

In the observational study, 12 samples were collected and MDR organisms were isolated, specifically from different wards of a tertiary care hospital. Stains isolated from the ICU were found to be the most resistant. From the Kirby-Bauer Sensitivity Test performed, it was observed that the majority of the antibiotics tested were not effective in inhibiting the growth of the bacterial strains. The study revealed that closed wards (ICU) acquired stains had higher AMR than those from the most exposed part (Ward, Reception, OPD) of the healthcare facility. I2 and I3 mostly retained their sensitivity against MDR stains. Furthermore, it was seen that the mutants of these bacteria started growing within a few hours. This suggests that the bacteria have developed genetic mutations that allow them to resist the effects of the antibiotics. The emergence of antibiotic-resistant strains of bacteria is a primary public health concern as it can make infections harder to treat and potentially more deadly. Policies promoting rational use of antibiotics, knowledge enhancement among prescribers and in the community, periodic ecology-based monitoring of AMR pattern, and antibiotic rotation can help in controlling infections and prevent the emergence of MDR nosocomial infections. The cost-effectiveness of such strategies must be analysed.

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