Screening of Multidrug-Resistant Bacteria Against β-Lactams Group of Antibiotics

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Abstract
The emergence of multidrug-resistant (MDR) bacteria has become a major challenge in the effective management of nosocomial infections. The present study focused on the isolation and identification of MDR bacteria collected from the various hospital environments, including surgical ward, maternity unit, toilet, and clinical waste materials. A total of 23 bacterial isolates were obtained from the mix culture as a pure culture. Morphological and biochemical characterization, including IMViC, catalase, and carbohydrate fermentation tests were performed for putative identification of the isolates.
Screening was done against selected standard β-lactam antibiotics, including Ampicillin (AMP, 10 µg), Ertapenem (ETP, 10 µg), Cefoxitin (CX, 30 µg), Cefixime (CFM, 5 µg), Ceftriaxone (CTR, 10 µg), Faropenem (FRP, 10 µg), Cefuroxime (CXM, 30 µg), Aztreonam (AT, 30 µg), and Amoxicillin-Clavulanic acid (AMC, 30 µg). Antibiotic susceptibility profiling was carried out using the Kirby-Bauer disc diffusion and agar well diffusion methods, which revealed that the majority of the isolates exhibited high levels of resistance to multiple generations of β-lactam antibiotics.
Among the 23 isolates, 09 bacterial isolates demonstrated 100% resistance to Amoxicillin, Cefuroxime, Faropenem, and Aztreonam. Furthermore, combination studies employing different antibiotic ratios (1:1, 1:3, and 3:1) failed to enhance antibacterial activity, indicating limited therapeutic effectiveness of these combinations. These findings highlight the widespread occurrence of MDR bacteria in hospital environments and emphasize the urgent need for alternative antimicrobial strategies to combat nosocomial infections.

Keywords: Multidrug resistance (MDR), Nosocomial infections, β-lactam antibiotics, and combinations.

Introduction

Antibiotic resistance has emerged as a serious global health challenge, severely limiting the treatment option for infectious diseases. In past, Multidrug-resistant (MDR) bacteria was limited to hospital settings, are now increasing in both clinical and community environments which underlines urgent surveillance and intervention strategies (WHO, 2023; Murray *et al.*, 2022). Major MDR bacteria includes Gram-negative pathogens such as *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia* and Gram-positive species such as *Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecium and Enterococcus faecalis* (CDC, 2025; WHO, 2023).
The most common antibiotic drugs are beta lactam antibiotics, which is included penicillin, cephalosporin, carbapenem, and monobactam. Through their interaction with penicillin-binding proteins, they prevent the formation of bacterial cell wall (Alfei *et al.,* 2022; Bush & Bradford, 2016; Drawz & Bonomo, 2010). Beta-lactamase enzymes hydrolyse the beta-lact am ring of beta lactam antibiotics and rendering the effect of medications inactive (Bush, 2018).
In healthcare settings, nosocomial infections remain a concern, results from prolonged hospitalization and spreading through contaminated surfaces, direct contact or medical equipment (Haque *et al.*, 2018; Weber *et al.,* 2010). Inadequate infection control practices among healthcare workers contribute significantly to transmission of hospital acquired infections (Allegranzi & Pittet, 2009, Kümmerer, 2009).
The present study focused on the isolation and screening of multidrug-resistant (MDR) bacteria obtained from the hospital environment, with a specific emphasis on their resistance profiles against different types of beta-lactam antibiotics.

Method & Materials

Sample Collection: Air samples were collected from various sections (maternity wards, surgical units, toilet area, diagnostic laboratories, common hall area) and discarded materials (pus and blood) were collected from pathology lab of hospital located the nearby Gwalior Madhya Pradesh. Collection and processing of samples

were done by following standard microbiological protocols. (Aneja., 2018).
Air samples were collected by placing nutrient agar petri plates at different mentioned collection sites. After 4 hours, the plates were placed in container containing ice packs to maintain the sample integrity and transported to the microbiology laboratory. Air samples were placed in incubator for the growth of bacteria. Blood and pus samples processing was initiated by serial dilution method followed by pouring and spread plate method.

Isolation and screening of bacterial isolates:

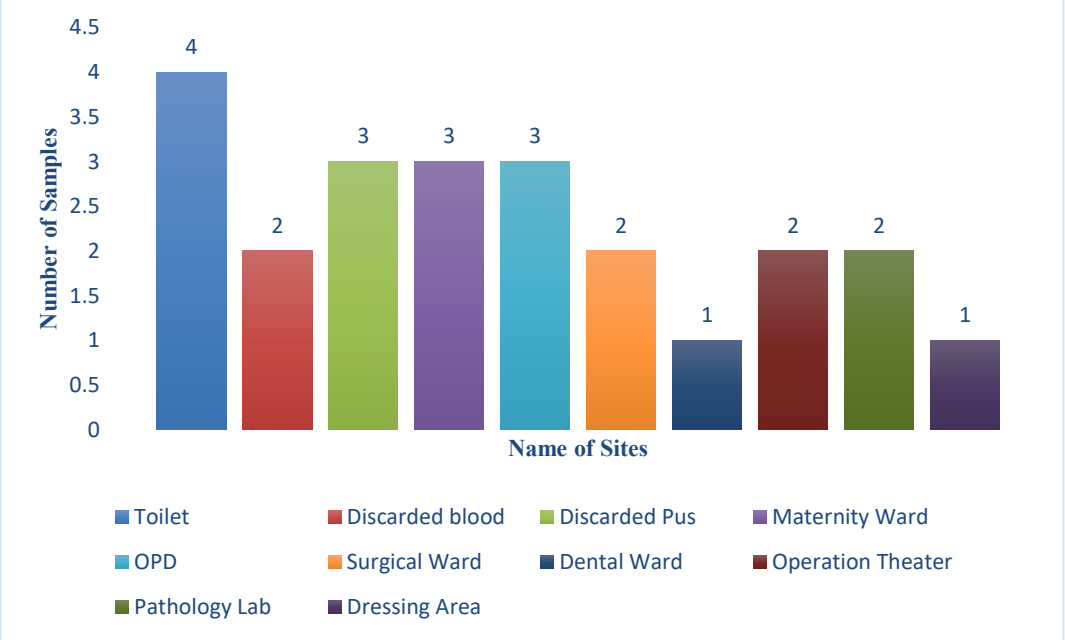
After 24 hrs. incubation, we obtained mixed bacterial cultures. Different colonies were selected from mixed cultures streaked onto nutrient agar media to obtain pure isolates. Gram staining and different standard biochemical assays (catalase activity, citrate utilization, indole production, methyl red-Voges-Proskauer (MR-VP) test, and sugar fermentation) were performed for the morphological and biochemical characterization (Aneja., 2018; Sharma *et al*., 2017; Sharma *et al.,* 2018).

Antibiotic-susceptibility Profiling

The antimicrobial sensitivity pattern of the isolate's bacteria was determined using the Kirby-Bauer disc diffusion method, following standard protocols. For the preparation of inoculum, 24 hrs old bacterial cultures were suspended in 4-5 mL of sterile distilled water, and the turbidity was adjusted to match the 0.5 McFarland standard, ensuring a uniform bacterial culture. This standardized inoculum was then evenly spread across the surface of Mueller-Hinton Agar (MHA) plates using a sterile cotton swab to achieve a confluent lawn of bacterial growth (Nathaniel et al., 2023).
After inoculation, commercially available antibiotic-impregnated discs were aseptically placed on the agar surface using sterile forceps. The antibiotic discs included Amoxicillin-Clavulanic acid (AMC, 30 µg) Ertapenem (ETP 10 µg), Cefoxitin (CX 30 µg), Cefixime (CXM 30 µg), Ceftriaxone (CTR 10 µg), Faropenem (FRP 10 µg), Cefuroxime (CFM 5 µg), and Aztreonam (AT, 30 µg).
The inoculated plates with antibiotic discs were incubated at 37 °C for 24 hours (Lee., et al 2019) to assess the sensitivity and resistance patterns.
Following the primary screening, the agar well diffusion method was employed to further confirm the antibiotic resistance patterns of the bacterial isolates. Antibiotics were prepared from commercially available tablets and injectable formulations, including amoxicillin, ertapenem, cefoxitin, cefixime, ceftriaxone, Faropenem, cefuroxime, aztreonam. Stock solutions of each antibiotic were prepared at a concentration of 1 mg/mL. For the combination study, four β-lactam antibiotics Aztreonam (AZT), Amoxicillin (AMX), Faropenem (FRP), and Cefuroxime (CXM) were selected to evaluate their synergistic antibacterial activity. The antibiotics were tested individually and in combinations at ratios of 1:1, 1:3, and 3:1. The zones of inhibition produced by the individual antibiotics and their combinations were measured and compared to determine whether the antibiotic combinations exhibited enhanced antibacterial activity (synergistic effect) against the bacterial isolates.
(Gideon., *et al* 2023; Lee., *et al* 2019).

Results and Discussion

In this study, a total no. of 23 pure bacterial isolates was obtained from the mixed cultures. The comprehensive approach for the sample ensures the inclusion of both environmental and clinical sources for a more representative analysis of bacterial isolates. (Komal *et al.,* 2025) Graph 1 and Table no. 1, represent the summary of sample collection sites with number of samples.



Graph 1: Graphical Summary of Sample Collection Sites

Table No. 1 Number of collected samples from different sections of hospital and discarded materials

S.No.	Toilet (A)	Discarded blood (B)	Discarded Pus (G)	Maternity Ward (D)	OPD (E)	Surgical ward (F)	Dental Ward (G)	Operation Theater (H)	Pathology lab (I)	Dressing Area (J)
1.	A1	B1	C1	D1	E1	F1	G1	H1	I1	J1
2..	A2	B2	C2	D2	E2	F2		H2	I2	
3.	A3		C3	D3	E3					
4.	A4									
	04	02	03	03	03	02	01	02	02	01
Total	23									

After obtaining the pure isolates, Bergey's Manual of Systematic Bacteriology were used for the morphological (table no. 2) and biochemical characterization (table no. 2) of 23 pure bacterial isolates (Dedysh *et al*., 2020).

Table no. – 2: Morphological Characterization of 23 pure bacterial isolates

S.NO	Cultures	Gram Staining	Morphology
1.	A1	Gram +ve	Bacilli
2.	A2	Gram –ve	Coccobacilli
3.	A3	Gram +ve	Bacilli
4.	A4	Gram -ve	Bacilli
5.	B1	Gram –ve	Bacilli
6.	B2	Gram +ve	Cocci
7.	C1	Gram +ve	Diplococcus
8.	C2	Gram –ve	Bacilli
9.	C3	Gram +ve	Cocci
10.	D1	Gram +ve	Cocci
11.	D2	Gram -ve	Bacilli
12.	D3	Gram +ve	Bacilli
13.	E1	Gram -ve	Bacilli
14.	E2	Gram -ve	Coccobacillus
15.	E3	Gram -ve	Coccobacillus
16.	F1	Gram +ve	Cocci
17.	F2	Gram +ve	Bacilli
18.	G1	Gram +ve	Monococcus
19.	H1	Gram -ve	Diplococcus
20.	H2	Gram -ve	Bacilli
21.	I1	Gram +ve	Monococcus
22.	I2	Gram +ve	Cocci
23.	J1	Gram -ve	Cocci

Among the 23 bacterial isolates, 12 were Gram-positive and 11 were Gram-negative. Based on their cellular morphology, the isolates were categorized into different form of bacilli, and cocci. The bacilli included isolates (A1, A3, A4, D2, E1, E3, H2 B2, C3, F3, G1); cocci included isolates (B1, C1, C4, D1, E2, I2, J1, C2, H1, I1).

Table No. -3: Biochemical Characterization of 23 pure bacterial isolates

S.no	Cultures	Indole Test	Methyl Red	Voges Proskauer	Citrate Utilization Test	Catalase Test
1.	A1	-	+	-	-	-
2.	A2	-	+	-	-	-
3.	A3	-	-	+	+	+
4.	A4	-	-	-	+	+
5.	B1	-	+	-	-	-
6.	B2	-	+	-	-	-
7.	C1	-	+	-	-	-
8.	C2	-	-	-	+	-
9.	C3	-	-	-	-	-
10.	D1	-	+	+	+	+
11.	D2	-	-	-	+	+
12.	D3	-	+	-	-	-
13.	E1	-	-	+	+	+
14.	E2	-	+	-	-	-
15.	E3	-	-	-	-	+
16.	F1	-	-	-	-	+
17.	F2	-	+	-	+	-
18.	G1	-	+	-	-	-
19.	H1	-	+	-	+	-
20.	H2	-	-	-	+	+
21.	I1	-	-	+	-	-
22.	I2	+	-	+	-	-
23.	J1	-	+	-	-	-

In biochemical analysis, it was observed Indole test showed negative for 22 bacterial isolates, where 01 was positive (Isolate - I2). The indole test is useful for differentiating enteric bacteria because it shows whether the organism can break down tryptophan to produce indole using the enzyme tryptophanase. This helps in identifying bacteria, as indole-positive organisms like *Escherichia coli* can be clearly distinguished from indole-negative members of the Enterobacteriaceae family. (Aneja., 2018)

In methyl red (MR) test, 12 were positive and 11 were negative out of 23 bacterial isolates. The methyl red test differentiates bacteria, particularly Enterobacteriaceae, by identifying those that produce large amounts of stable acid during glucose fermentation (Aneja., 2018).

In vogues Proskauer test, 5 were positive and 18 were negative out of 23 bacterial isolates. The VP test detects the ability of bacteria to metabolize the pyruvate into a neutral intermediate product called 'acetylmethylcarbinol' or 'acetoin (Aneja., 2018).

In citrate utilization test, 09 were gives positive and 14 were observed negative out of 23 bacterial isolates. This test differentiates Gram-negative bacteria based on their ability to metabolize citrate, often used to differentiate Enterobacteriaceae members (e.g., *Klebsiella* is positive, *E. coli* is negative) (Aneja., 2018).

In catalase test, 8 were positive and 15 were negative. It detects the enzyme catalase, which decomposes toxic hydrogen peroxide into water and oxygen (Aneja., 2018).

Carbohydrate fermentation tests were performed on all 23 bacterial isolates to evaluate their ability to utilize different carbohydrates as carbon sources. The results showed that the majority of the isolates were able to ferment sucrose, dextrose, and lactose. Among the 23 isolates, all utilized sucrose except isolates B2, C4, D2, F2, and F3. For dextrose fermentation, isolates B1, C4, E2, F1, F2, and F3 showed negative results, indicating their inability to utilize dextrose. Similarly, lactose fermentation was negative for isolates A2, B2, C1, C2, C3, C4, D1, D2, E2, F1, F2, F3, I1, and J1, suggesting that these isolates could not utilize lactose for growth (Aneja, 2018). The complete carbohydrate fermentation profile of all isolates is presented in (Table 4).

Table no. – 4: Summary of Carbohydrate fermentation for 23 pure bacterial isolates

S.NO	Cultures	Sucrose	Dextrose	Lactose
1.	A1	+++	+++	+++
2.	A2	+++	+++	-
3.	A3	+++	+++	+++
4.	A4	+	-	+
5.	B1	-	+	-
6.	B2	+	+	-
7.	C1	++	+	-

8.	C2	+	+	-
9.	C3	-	-	-
10.	D1	++	+	-
11.	D2	-	+	-
12.	D3	+	+	+
13.	E1	+	-	-
14.	E2	+	-	-
15.	E3	-	-	-
16.	F1	-	-	-
17.	F2	++	++	+
18.	G1	++	+	+
19.	H1	++	+	+
20.	H2	++	+	+
21.	I1	++	++	-
22.	I2	++	++	++
23.	J1	++	++	-

(+++) Strong, (++) Moderate, (+) Weak, (-) No Growth

After morphological and biochemical analysis, results were used for the putative classification of 23 bacterial isolates (Table no.5).

Table No. – 5: putative classification of 23 bacterial isolates

S. No.	Samples No.	Putative Identity of Isolates
1.	A1, A3, A4, D2, E1, E3, H2 B2, C3, F3, G1	*Bacillus* Species
2.	A2	*Enterobacter* Species
3.	B1, C1, C4, D1, E2, F1, I2, J1	*Staphylococcus* Species
4.	C2, H1, I1	*Streptococcus* Species

All bacterial isolates were used for antibacterial sensitivity test. It was done by two methods - disc diffusion and agar well diffusion, (Singh, *et al.,* 2020), followed the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI,2020). It was used to categorizing the bacterial isolates as sensitive, intermediate, or resistant.

Table no. – 6 and 7 is showing the resistant, sensitive and Intermediate profiling of 23 bacterial isolates. Isolates exhibiting resistance to two or more distinct classes of antibiotics were classified as multidrug-resistant (MDR) (CLSI., 2020; Komal *et al.,* 2025). The ability of genetic adaptability by bacteria has highlighted the global spread of MDR with increased morbidity, mortality and healthcare burden among the population (Munita & Arias, 2016; Prestinaci *et al.,* 2015). Antibacterial activity of different antibiotics were presented in table no 6 using disc diffusion method

Table No. – 6: Antibacterial Activity Based on The Disc Diffusion Method

S. No	Cultures	Antibiotic Discs	Zone of Inhibition in mm	Resistant/ Sensitive	Remarks
1.	A1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	26mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	17mm	R	
		Cefixime (CXM 30)	-	R	Resistant-5
		Ceftriaxone (CTR 10)	19mm	S	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
2.	A2	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	23mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	21mm	S	
		Cefixime (CXM 30)	16mm	S	Resistant-4
		Ceftriaxone (CTR 10)	17mm	S	Sensitive-4
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	

3.	A3	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	-	R	Antibiotic Discs:
		Cefoxitin (CX30)	-	R	
		Cefixime (CXM 30)	-	R	Resistant-8
		Ceftriaxone (CTR 10)	-	R	Sensitive-0
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
4.	A4	Amoxicillin (AMX10)	-	R	
		Ertapenem (ETP 10)	-	R	Resistant-6
		Cefoxitin (CX30)	-	R	Sensitive-2
		Cefixime (CXM 30)	-	R	Intermediate - 0
		Ceftriaxone (CTR 10)	11mm	R	
		Faropenem (FRP 10)	10mm	I	
		Cefuroxime (CFM 5)	-	I	
		Aztreonam (AT 30)		R	
5.	B1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	26mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	18mm	S	
		Cefixime (CXM 30)	-	R	Resistant-1
		Ceftriaxone (CTR 10)	20mm	S	Sensitive-6
		Faropenem (FRP 10)	17mm	S	Intermediate - 1
		Cefuroxime (CFM 5)	12mm	R	
		Aztreonam (AT 30)	-	R	
6.	B2	Amoxicillin (AMX10)	16mm	S	Among 8
		Ertapenem (ETP 10)	18mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	19mm	S	
		Cefixime (CXM 30)	29mm	S	Resistant-1
		Ceftriaxone (CTR 10)	22mm	S	Sensitive-6
		Faropenem (FRP 10)	19mm	S	Intermediate - 1
		Cefuroxime (CFM 5)	10mm	I	
		Aztreonam (AT 30)	-	R	
7.	C1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	20mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	19mm	S	
		Cefixime (CXM 30)	16mm	S	Resistant-5
		Ceftriaxone (CTR 10)	-	R	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
8.	C2	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	17mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	19mm	S	
		Cefixime (CXM 30)	14mm	S	Resistant-5
		Ceftriaxone (CTR 10)	-	R	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
9.	C3	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	-	R	Antibiotic Discs:
		Cefoxitin (CX30)	16mm	S	
		Cefixime (CXM 30)	18mm	S	Resistant-5
		Ceftriaxone (CTR 10)	19mm	S	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
10.	D1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	21mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	17mm	S	
		Cefixime (CXM 30)	19mm	S	Resistant-6
		Ceftriaxone (CTR 10)	21mm	S	Sensitive-1
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	

11.	D2	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	17mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	16mm	S	
		Cefixime (CXM 30)	19mm	S	Resistant-4
		Ceftriaxone (CTR 10)	12mm	I	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 1
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
12.	D3	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	17mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	16mm	S	
		Cefixime (CXM 30)	19mm	S	Resistant-4
		Ceftriaxone (CTR 10)	21mm	S	Sensitive-4
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
13.	E1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	-	S	Antibiotic Discs:
		Cefoxitin (CX30)	-	S	
		Cefixime (CXM 30)	-	R	Resistant-5
		Ceftriaxone (CTR 10)	19mm	S	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
14.	E2	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	18mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	19mm	S	
		Cefixime (CXM 30)	26mm	S	Resistant-5
		Ceftriaxone (CTR 10)	-	R	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
15.	E3	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	25mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	13mm	I	
		Cefixime (CXM 30)	-	R	Resistant-5
		Ceftriaxone (CTR 10)	20mm	S	Sensitive-2
		Faropenem (FRP 10)	-	R	Intermediate - 01
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
16.	F1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	22mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	19mm	S	
		Cefixime (CXM 30)	-	R	Resistant-5
		Ceftriaxone (CTR 10)	16mm	S	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
17.	F2	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	-	R	Antibiotic Discs:
		Cefoxitin (CX30)	17mm	S	
		Cefixime (CXM 30)	-	R	Resistant-6
		Ceftriaxone (CTR 10)	19mm	S	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
18.	G1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	22mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	11mm	I	
		Cefixime (CXM 30)	12mm	I	Resistant-3
		Ceftriaxone (CTR 10)	15mm	S	Sensitive-2
		Faropenem (FRP 10)	13mm	I	Intermediate - 03
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	

19	H1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	25mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	19mm	S	
		Cefixime (CXM 30)	23mm	S	Resistant-3
		Ceftriaxone (CTR 10)	21mm	S	Sensitive-4
		Faropenem (FRP 10)	14mm	I	Intermediate - 01
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
20	H2	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	-	R	Antibiotic Discs:
		Cefoxitin (CX30)	20mm	S	
		Cefixime (CXM 30)	13mm	I	Resistant-4
		Ceftriaxone (CTR 10)	11mm	I	Sensitive-1
		Faropenem (FRP 10)	12mm	I	Intermediate - 03
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	
21.	I1	Amoxicillin (AMX10)	11mm	I	Among 8
		Ertapenem (ETP 10)	21mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	13mm	I	
		Cefixime (CXM 30)	20mm	S	Resistant-2
		Ceftriaxone (CTR 10)	14mm	I	Sensitive-2
		Faropenem (FRP 10)	-	R	Intermediate - 04
		Cefuroxime (CFM 5)	10mm	I	
		Aztreonam (AT 30)	-	R	
22.	I2	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	22mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	17mm	S	
		Cefixime (CXM 30)	-	R	Resistant-5
		Ceftriaxone (CTR 10)	16mm	S	Sensitive-3
		Faropenem (FRP 10)	-	R	Intermediate - 0
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30))	-	R	
23.	J1	Amoxicillin (AMX10)	-	R	Among 8
		Ertapenem (ETP 10)	21mm	S	Antibiotic Discs:
		Cefoxitin (CX30)	17mm	S	
		Cefixime (CXM 30)	10mm	I	Resistant-5
		Ceftriaxone (CTR 10)	-	R	Sensitive-2
		Faropenem (FRP 10)	-	R	Intermediate - 01
		Cefuroxime (CFM 5)	-	R	
		Aztreonam (AT 30)	-	R	

For agar well diffusion (Table no. 7), screening was performed using β-lactam antibiotics (Amoxicillin, Ertapenem, Cefoxitin, Cefixime, Ceftriaxone, Faropenem, Cefuroxime, Aztreonam) at a concentration of 1 mg/mL. Antibiotic susceptibility testing was conducted to determine the resistance patterns of all isolates. The results demonstrated that all tested antibiotics were ineffective against the isolates, indicating a high level of resistance (Gideon *et al.,* 2023).

Table no. – 7: Antibacterial Activity of Based on Agar Well Diffusion

S. No	Cultures	Antibiotics	Zone of Inhibition in mm	Resistant/ Sensitive	Remarks
1.	A1	Amoxicillin	-	R	Sensitive -2
		Ertapenem	15mm	S	Resistant – 6
		Cefoxitin	-	R	Intermediate - 1
		Cefixime	19mm	S	
		Ceftriaxone	14mm	I	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	

2.	A2	Amoxicillin	-	S	Sensitive – 3
		Ertapenem	19mm	S	Resistant – 5
		Cefoxitin	22mm	R	Intermediate - 0
		Cefixime	17mm	S	
		Ceftriaxone	-	R	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	
3.	A3	Amoxicillin	-	R	Among 8
		Ertapenem	-	R	antibiotic discs:
		Cefoxitin	-	R	
		Cefixime	-	R	Resistant-8
		Ceftriaxone	-	R	Sensitive-0
		Faropenem	-	R	Intermediate - 0
		Cefuroxime	-	R	
		Aztreonam	-	R	
4.	A4	Amoxicillin	-	R	Among 8
		Ertapenem	-	R	antibiotic discs:
		Cefoxitin	-	R	
		Cefixime	-	R	Resistant-8
		Ceftriaxone	-	R	Sensitive-0
		Faropenem	-	R	Intermediate - 0
		Cefuroxime	-	R	
		Aztreonam	-	R	
5.	B1	Amoxicillin	-	R	Among 8
		Ertapenem	21mm	S	antibiotic discs:
		Cefoxitin	18mm	S	
		Cefixime	20mm	S	Resistant-4
		Ceftriaxone	19mm	S	Sensitive-4
		Faropenem	-	R	Intermediate - 0
		Cefuroxime	-	R	
		Aztreonam	-	R	
6.	B2	Amoxicillin	-	R	Sensitive – 4
		Ertapenem	16mm	S	Resistant – 4
		Cefoxitin	20mm	S	Intermediate - 0
		Cefixime	18mm	S	
		Ceftriaxone	19mm	S	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	
7.	C1	Amoxicillin	-	R	Sensitive – 4
		Ertapenem	17mm	S	Resistant – 3
		Cefoxitin	23mm	S	intermediate - I
		Cefixime	22mm	S	
		Ceftriaxone	16mm	S	
		Faropenem	-	R	
		Cefuroxime	10mm	I	
		Aztreonam	-	R	
8.	C2	Amoxicillin	-	R	Sensitive – 3
		Ertapenem	21mm	S	Resistant – 5
		Cefoxitin	19mm	S	Intermediate - 0
		Cefixime	22mm	S	
		Ceftriaxone	-	R	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	
9.	C3	Amoxicillin	-	R	Sensitive – 1
		Ertapenem	-	R	Resistant – 7
		Cefoxitin	-	R	Intermediate - 0
		Cefixime	17mm	S	
		Ceftriaxone	-	R	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	

10.	D1	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime Aztreonam	- - - - 22mm - - -	R R R R S R R R	Sensitive – 1 Resistant – 7 Intermediate – 0
11.	D2	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime Aztreonam	- - - 22mm 13mm - - -	R R R S I R R R	Sensitive – 1 Resistant – 6 Intermediate – 01
12.	D3	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime Aztreonam	21mm 23mm - 19mm 21mm 18mm - -	S S R S S S R R	Among 8 Antibiotics Sensitive – 6 Resistant – 2 Intermediate - 0
13.	E1	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime Aztreonam	- - - 15mm 21mm - - -	R R R S S R R R	Among 8 antibiotics Sensitive – 2 Resistant – 6 Intermediate – 0
14.	E2	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime Aztreonam	- - - - - - - -	R R R R R R R R	Among - 8 antibiotics Resistant- 8 Sensitive - 0 Intermediate - 0
15.	E3	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime Aztreonam	- - 20mm - 19mm - - -	R R S R S R R R	Sensitive – 2 Resistant – 6 Intermediate – 0
16.	F1	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime Aztreonam	- 19mm - - - 10mm - -	R S R R R I R R	Sensitive – 1 Resistant – 6 Intermediate – 1
17.	F2	Amoxicillin Ertapenem Cefoxitin Cefixime Ceftriaxone Faropenem Cefuroxime	- 16mm - 26mm 15mm - -	R S R S S R R	Sensitive – 5 Resistant – 3 Intermediate – 0

		Aztreonam	-	R	
18.	G1	Amoxicillin	-	R	Sensitive – 5
		Ertapenem	19mm	S	Resistant – 3
		Cefoxitin	21mm	S	Intermediate – 0
		Cefixime	-	R	
		Ceftriaxone	17mm	S	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	
19.	H1	Amoxicillin	-	R	Sensitive – 2
		Ertapenem	17mm	S	Resistant – 6
		Cefoxitin	-	R	Intermediate – 0
		Cefixime	16mm	S	
		Ceftriaxone	-	R	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	
20.	H2	Amoxicillin	-	R	Among 10
		Ertapenem	-	R	antibiotic discs:
		Cefoxitin	-	R	
		Cefixime	-	R	Resistant-8
		Ceftriaxone	-	R	Sensitive-0
		Faropenem	-	R	Intermediate - 0
		Cefuroxime	-	R	
		Aztreonam	-	R	
21.	I1	Amoxicillin	-	R	Sensitive – 2
		Ertapenem	16mm	S	Resistant – 8
		Cefoxitin	17mm	S	Intermediate - 0
		Cefixime	19mm	S	
		Ceftriaxone	20mm	S	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	
22.	I2	Amoxicillin	-	S	Sensitive – 6
		Ertapenem	19mm	S	Resistant – 4
		Cefoxitin	-	R	Intermediate – 0
		Cefixime	16mm	S	
		Ceftriaxone	-	R	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	
23.	J1	Amoxicillin	-	R	Sensitive – 3
		Ertapenem	16mm	S	Resistant – 5
		Cefoxitin	-	R	Intermediate - 0
		Cefixime	17mm	S	
		Ceftriaxone	16mm	S	
		Faropenem	-	R	
		Cefuroxime	-	R	
		Aztreonam	-	R	

We have observed 09 cultures were showed 100% resistance against Amoxicillin, Cefuroxime, Faropenem, and Aztreonam.

Screening of MDR bacterial isolates and ineffective antibiotics were further confirmed by synergistic activity of different antibiotics, namely Aztreonam (AZT), Amoxicillin (AMX), Faropenem (FRP), and Cefuroxime (CXM). The antibiotics were tested in different ratios (1:1, 1:3, and 3:1) to determine whether their combinations were enhanced antibacterial activity or not. The results showed that the efficacy of the antibiotic combinations varied among the bacterial isolates. Some combinations and ratios produced a clear zone of inhibition (ZOI), indicating antibacterial activity, whereas others showed complete resistance with no zone of inhibition. These findings, summarized in Table no. 8. The inconsistent response observed in the present study highlights the need to explore alternative therapeutic approaches, such as drug repurposing and nanoparticle-based antimicrobial strategies, to combat multidrug-resistant hospital-acquired infections.

Table 8. Synergistic Activity of Selected Antibiotic Combinations

S. No	Name of Cultures	Name of combination 1mg/ml	Ratio 1:1		1:3		3:1	
			ZOI	R/S	ZOI	R/S	ZOI	R/S
1	A1	AZT+ AMX	15mm	S	-	R	-	R
		AZT+FRP	15mm	S	-	R	-	R
		AZT+CXM	16mm	S	-	R	14mm	I
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	15mm	S	-	R	-	I
		FRP+CXM	-	R	-	R	-	R
2.	A2	AZT+ AMX	-	R	10mm	I	-	R
		AZT+FRP	-	R	16mm	S	15mm	S
		AZT+CXM	-	R	10mm	I	11mm	I
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
3.	A3	AZT+ AMX	<10mm	I	12mm	I	11mm	I
		AZT+FRP	13mm	S	10mm	I	-	R
		AZT+CXM	12mm	I	11mm	I	12mm	I
		AMX+FRP	-	R	-	R	-	
		AMX+CXM	-	R	-	R	14mm	I
		FRP+CXM	-	R	-	R	-	R
4.	A4	AZT+ AMX	-	R	-	R	-	R
		AZT+FRP	-	R	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
5.	B1	AZT+ AMX	15mm	S	-	R	16mm	S
		AZT+FRP	16mm	S	10mm	I	17mm	S
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
6.	B2	AZT+ AMX	16mm	S	-	R	18mm	S
		AZT+FRP	17mm	S	-	R	19mm	S
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
7.	C1	AZT+ AMX	16mm	S	14mm	I	17mm	S
		AZT+FRP	17mm	S	14mm	I	17mm	S
		AZT+CXM	-	R	-	R	18mm	S
		AMX+FRP	14mm	I	-	R	-	R

		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
8.	C2	AZT+ AMX	-	R	10mm	I	-	R
		AZT+FRP	-	R	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
9.	C3	AZT+ AMX	14mm	I	10mm	I	10mm	I
		AZT+FRP	13mm	I	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
10.	D1	AZT+ AMX	-	R	-	R	-	R
		AZT+FRP	-	R	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
11.	D2	AZT+ AMX	-	R	-	R	-	R
		AZT+FRP	-	R	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
12.	D3	AZT+ AMX	16mm	S	-	R	17mm	S
		AZT+FRP	17mm	S	19mm	S	19mm	S
		AZT+CXM	16mm	S	15mm	R	18mm	S
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	19mm	S	-	R
13.	E1	AZT+ AMX	-	R	-	R	-	R
		AZT+FRP	13mm	I	14mm	I	14mm	I
		AZT+CXM	14mm	I	-	R	10mm	I
		AMX+FRP	10mm	I	-	R	-	R
		AMX+CXM	-	R	-	R	10mm	I
		FRP+CXM	-	R	-	R	15mm	S
14.	E2	AZT+ AMX	-	R	-	R	-	R
		AZT+FRP	-	R	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
15.	E3	AZT+ AMX	16mm	S	-	R	17mm	S
		AZT+FRP	-	R	-	R	13mm	I
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R

16.	F1	AZT+ AMX	-	R	-	R	-	R
		AZT+FRP	-	R	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
17.	F2	AZT+ AMX	-	R	17mm	S	16mm	S
		AZT+FRP	-	R	16mm	S	18mm	S
		AZT+CXM	-	R	15mm	S	12mm	I
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
18.	G1	AZT+ AMX	17mm	S	-	R	-	R
		AZT+FRP	18mm	S	19mm	S	19mm	S
		AZT+CXM	-	R	10mm	I	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
19.	H1	AZT+ AMX	16mm	S	14mm	I	19mm	S
		AZT+FRP	17mm	S	10mm	I	17mm	S
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
20.	H2	AZT+ AMX	14mm	I	-	R	11mm	I
		AZT+FRP	13mm	I	-	R	13mm	I
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
21.	I1	AZT+ AMX	16mm	S	-	R	-	R
		AZT+FRP	-	R	-	R	-	R
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
22.	I2	AZT+ AMX	16mm	S	16mm	S	18mm	S
		AZT+FRP	17mm	S	-	R	19mm	S
		AZT+CXM	-	R	-	R	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R
23.	J1	AZT+ AMX	20mm	S	-	R	18mm	S
		AZT+FRP	22mm	S	17mm	S	19mm	S
		AZT+CXM	-	R	19mm	S	-	R
		AMX+FRP	-	R	-	R	-	R
		AMX+CXM	-	R	-	R	-	R
		FRP+CXM	-	R	-	R	-	R

After the combinations study, the resistant pattern obtained from disc diffusion method, well diffusion method

and synergistic activity of selected antibiotics, it was observed that 9 bacterial isolates (A3, A4, D1, D2, E1, E2, E3, F1, H2) were completely showed resistance, whereas 14 (A1, A2, B1, B2, C1, C2, C3, D3, F2, G1, H1, I1, I2, J1) bacterial isolates showed intermediate and sensitivity.

Increasing resistant patterns is highlighting the major issues among the bacteria in hospital facility. Development of multidrug resistance in bacteria is caused by the acquisition of mobile genetic elements which carries resistance genes and transferred by the process of horizontal gene exchanges (Davies & Davies, 2010). Bacteria involve various mechanisms such as efflux pump activation, reduced membrane permeability, enzymatic degradation, and target modification to evade antimicrobial action (Blair *et al.,* 2015). These processes are triggered by antibiotic misuse, dependence on broad-spectrum drugs as well as limited availability of targeted therapeutics (Ventola, 2015; WHO, 2023).

Conclusion

In this study, bacterial isolates were screened from different section of hospital including air and discarded material. Findings revealed that these bacterial isolates exhibited complete resistance against the amoxicillin, faropenam, cefuroxime, aztreonam. The present study demonstrated the widespread occurrence of multidrug-resistant (MDR) bacterial isolates in hospital environments. Such resistance pattern highlights the alarming adaptability of these pathogens and underscores the urgent need for novel antimicrobial strategies and robust infection control measures.

Furthermore, combination and synergistic effect of beta lactam antibiotics did not improve antibacterial efficiency. In order to reduce the increasing risk of MDR associated infections, these findings collectively highlighted the critical need for ongoing infection control procedure and the creation of alternate therapeutic approaches.

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References

1. Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology, 13(1), 42–51. https://doi.org/10.1038/nrmicro3380
2. Centers for Disease Control and Prevention. (2022). Antibiotic resistance threats in the United States. https://www.cdc.gov/drugresistance/biggest-threats.html
3. Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74(3), 417–433. https://doi.org/10.1128/MMBR.00016-10
4. Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0
5. Ventola, C. L. (2015). The antibiotic resistance crisis: Causes and threats. Pharmacy and Therapeutics, 40(4), 277–283.
6. World Health Organization. (2023). Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
7. Bush, K. (2018). Past and present perspectives on β-lactamases. Antimicrobial Agents and Chemotherapy, 62(10), e01076-18. https://doi.org/10.1128/AAC.01076-18
8. Bush, K., & Bradford, P. A. (2016). β-Lactams and β-lactamase inhibitors: An overview. Cold Spring Harbor Perspectives in Medicine, 6(8), a025247. https://doi.org/10.1101/cshperspect.a025247
9. Drawz, S. M., & Bonomo, R. A. (2010). Three decades of β-lactamase inhibitors. Clinical Microbiology Reviews, 23(1), 160–201. https://doi.org/10.1128/CMR.00037-09
10. Kong, K. F., Schneper, L., & Mathee, K. (2010). Beta-lactam antibiotics: From antibiosis to resistance and bacteriology. APMIS, 118(1), 1–36. https://doi.org/10.1111/j.1600-0463.2009.02563.x
11. Ambler, R. P. (1980). The structure of beta-lactamases. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 289(1036), 321–331. https://doi.org/10.1098/rstb.1980.0049
12. Bush, K., & Jacoby, G. A. (2010). Updated functional classification of β-lactamases. Antimicrobial Agents and Chemotherapy, 54(3), 969–976. https://doi.org/10.1128/AAC.01009-09
13. Queenan, A. M., & Bush, K. (2007). Carbapenemases: The versatile β-lactamases. Clinical Microbiology Reviews, 20(3), 440–458. https://doi.org/10.1128/CMR.00001-07
14. Chartier, Y., Emmanuel, J., Pieper, U., Prüss, A., Rushbrook, P., Stringer, R., Townend, W., Wilburn, S., & Zghondi, R. (2014). Safe management of wastes from health-care activities (2nd ed.). World Health Organization.
15. Haque, M., Sartelli, M., McKimm, J., & Abu Bakar, M. (2018). Health care-associated infections – an overview. Infection and Drug Resistance, 11, 2321–2333. https://doi.org/10.2147/IDR.S177247
16. World Health Organization. (2011). Report on the burden of endemic health care-associated infection worldwide. WHO Press.
17. World Health Organization. (2014). Safe management of wastes from health-care activities (2nd ed.). WHO Press

18. Allegranzi, B., & Pittet, D. (2009). Role of hand hygiene in healthcare-associated infection prevention. Journal of Hospital Infection, 73(4), 305–315. https://doi.org/10.1016/j.jhin.2009.04.019
19. Centers for Disease Control and Prevention (CDC). (2020). Guidelines for environmental infection control in health-care facilities. https://www.cdc.gov/infectioncontrol
20. Otter, J. A., Yezli, S., Salkeld, J. A. G., & French, G. L. (2013). Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. American Journal of Infection Control, 41(5), S6–S11. https://doi.org/10.1016/j.ajic.2012.12.004
21. Weber, D. J., Rutala, W. A., Miller, M. B., Huslage, K., & Sickbert-Bennett, E. (2010). Role of hospital surfaces in the transmission of emerging health care-associated pathogens. American Journal of Infection Control, 38(5), S25–S33. https://doi.org/10.1016/j.ajic.2010.04.196
22. World Health Organization (WHO). (2011). Report on the burden of endemic health care-associated infection worldwide. WHO Press.
23. Bush, K., & Bradford, P. A. (2020). Epidemiology of β-lactamase-producing pathogens. Clinical Microbiology Reviews, 33(2), e00047-19. https://doi.org/10.1128/CMR.00047-19
24. Kümmerer, K. (2009). Antibiotics in the aquatic environment – A review – Part I. Chemosphere, 75(4), 417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086
25. Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., … Cars, O. (2013). Antibiotic resistance—the need for global solutions. The Lancet Infectious Diseases, 13(12), 1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9
26. Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. Microbiology Spectrum, 4(2), 1–37. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015
27. Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. Pathogens and Global Health, 109(7), 309–318. https://doi.org/10.1179/2047773215Y.0000000030
28. Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. P & T, 40(4), 277–283.
29. World Health Organization. (2020). Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
30. Fawole M.O. and Oso B.A., Laboratory manual of microbiology, Spectrum book Limited Ibadan, Nigeria, 123 (2007) for methodology
31. Aneja K.R., Laboratory manual of microbiology and biotechnology, ED-TECH (2018).
32. Sharma N., Jana A.M., Pathak N., Singh C. and Singh P., A preliminary study on the antibacterial effects of ethanolic extract of Psidium guajava leaves on Bacteria isolated from urinary tract infection with special reference to Escherichia coli and Staphylococcus aureus, International Journal of Biotechnology and Biochemistry, 13(2), 183-9 (2017)
33. Sharma N. and Rathore D.S., Antibacterial effects of Citrus limon peel extract on human pathogenic bacteria with special reference to Urinary Tract Infection, Int. J. Sci. Res. in Biological Sciences, 5(2), 27-32 (2018).
34. Nathaniel E., Ikram J., James A., Obaid B., Zahid A., Ahmed Z., Wazir D.K., Wahab Q.M., Varrassi G., Kumar S. and Khatri M., Molecular Characterization and Antibiotic Susceptibility Pattern of Bacterial Strains Isolated from Wound of Patients with Diabetes, Cureus, 15(10), e47681 (2023).
35. Gideon, M., & Ladan, Z. (2023). Synergistic combinatorial strategy for combating Antimicrobial Resistance (AMR) in clinical bacteria by combining antibiotics with plant extracts. Fine Chemical Engineering, 1-12.
36. Lee, Y. C., Chen, P. Y., Wang, J. T., & Chang, S. C. (2019). A study on combination of daptomycin with selected antimicrobial agents: in vitro synergistic effect of MIC value of 1 mg/L against MRSA strains. BMC Pharmacology and Toxicology, 20(1), 25.
37. Humphries, R. M., Ambler, J., Mitchell, S. L., Castanheira, M., Dingle, T., Hindler, J. A., Koeth, L., Sei, K., & CLSI Methods Development and Standardization Working Group of the Subcommittee on Antimicrobial Susceptibility Testing (2018). CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests. Journal of clinical microbiology, 56(4), e01934-17. https://doi.org/10.1128/JCM.01934-17.
38. Fakruddin M., Sultana R., Rahaman M.M., Hossain M.N. and Morshed M., Comparative study of different methds of genomic DNA extraction from Betel leaf (Piper betle L.) for detection of Salmonella spp., Bangladesh J. Ind. Microbiol. Biotechnol., 1(1), 20-8 (2017).
39. Komal, K., Neha, S., & Singh, T. R. (2025). Molecular Characterization and Phylogenetic Analysis of Multidrug Resistant Microorganism with Special Reference to Nosocomial Infections. Research Journal of Biotechnology Vol, 20, 7.
40. Starkova, D., Egorova, S., Suzhaeva, L., Nguyen, T. Q., Kaftyreva, L., Makarova, M., Zhamborova, S., Polev, D., Saitova, A., Nguyen, V. H., Vo, T. K., & Nguyen, L. T. (2025). Antimicrobial Resistance and Phylogenetic Analysis of Multidrug-Resistant Non-Typhoidal Salmonella Isolates from Different Sources in Southern Vietnam. Antibiotics (Basel, Switzerland), 14(5), 489. https://doi.org/10.3390/antibiotics14050489
41. Alfei, S., & Schito, A. M. (2022). β-lactam antibiotics and β-lactamase enzymes inhibitors, part 2: our limited resources. Pharmaceuticals, 15(4), 476.
42. Singh, S., Ahmad, S., Mehta, D., & Alam, S. (2020). Antibacterial activity in pyrimidine derivatives in lab, using agar well-diffusion method-an update on novel research. Pharm. Sci. Technol., 3, 40.
43. Dedysh, S. N., Losey, N. A., & Lawson, P. T. (2020). Bergey's manual of systematics of archaea and bacteria. *Whitman, WB, Rainey, FA, Kämpfer, P., Trujillo, ME, DeVos, P., Hedlund, B., Dedysh, S., Eds*, 1-2.