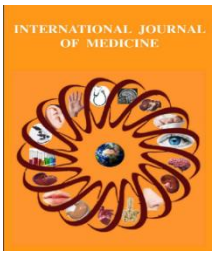

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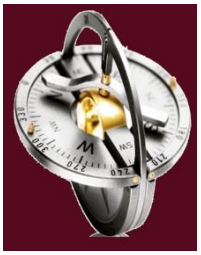

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Original Research

Pattern of Multidrug-Resistant Bacterial Isolates from Clinical Samples: A Hospital-Based Observational Study

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Abstract

Background: Multidrug-resistant bacterial infections restrict empirical treatment options and increase treatment difficulty. Local surveillance is essential for rational antibiotic policy. *Objectives:* To assess the distribution of bacterial isolates from clinical samples and determine the pattern of multidrug resistance in a hospital-based observational study. *Methods:* This study was conducted at Maheshwara Medical College, Patancheru, Hyderabad, Telangana, India, from July 2023 to December 2024. A total of 100 non-duplicate bacterial isolates from urine, pus/wound swabs, respiratory samples, blood, body fluids, and catheter-related samples were included. Organisms were identified by routine microbiological methods. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion, and multidrug resistance was defined as resistance to at least one antimicrobial agent in three or more antimicrobial categories. Data were analysed using frequencies and percentages. *Results:* Gram-negative organisms accounted for 76.0% of isolates, while Gram-positive organisms constituted 24.0%. Urine was the commonest sample source. Escherichia coli was the leading isolate, followed by Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Acinetobacter baumannii. Overall MDR prevalence was 64.0%. MDR was highest in Acinetobacter baumannii, followed by Klebsiella pneumoniae and Escherichia coli. Among Gram-negative isolates, resistance was highest to ampicillin/amoxicillin-clavulanate, ceftriaxone, ceftazidime, and ciprofloxacin. Among Gram-positive isolates, resistance was highest to penicillin, erythromycin, and ciprofloxacin. ESBL production was detected in 62.5% of Enterobacterales, carbapenem resistance in 21.1% of Gram-negative bacilli, and MRSA in 41.7% of Staphylococcus aureus. *Conclusion:* The study demonstrated a high MDR burden, particularly among Gram-negative bacilli. Routine antibiogram surveillance, antimicrobial stewardship, and infection-control practices are required to guide empirical therapy and restrict further resistancet.

Keywords: *Multidrug resistance; antimicrobial resistance; bacterial isolates; clinical samples; ESBL; MRSA; hospital-based observational study.*

Introduction

Antimicrobial resistance has become one of the most serious threats to modern clinical practice. Bacterial pathogens that were previously responsive to commonly used antibiotics are now increasingly resistant to multiple drug classes, resulting in delayed clinical response, escalation to reserve antibiotics, longer hospitalisation, and higher treatment cost. The global burden analysis of bacterial antimicrobial resistance highlighted its major contribution to mortality, particularly in low- and middle-income settings where infectious disease burden and antimicrobial pressure remain high [1]. The drivers of resistance are complex and include irrational antibiotic use, incomplete treatment, over-the-counter access, inadequate infection-control practices, and movement of resistant clones between community and hospital environments [2].

Hospital-based surveillance is a practical approach for understanding local antimicrobial resistance trends. Tertiary-care hospitals receive patients with severe infections, prior antibiotic exposure, invasive device use, and repeated healthcare contact, making them important reservoirs for multidrug-resistant organisms. Health-care-associated infections remain a substantial problem in developing countries, and Gram-negative bacilli have been repeatedly identified as important nosocomial pathogens in such settings [3]. Local antibiograms are therefore essential because resistance patterns differ across hospitals, wards, patient groups, and time periods. Empirical antibiotic protocols based only on national or international data often fail to reflect the actual resistance burden in a given institution.

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Multidrug resistance is commonly defined as non-susceptibility to at least one agent in three or more antimicrobial categories [4]. This definition provides a standard framework for comparing bacterial resistance across studies and laboratories. Among Gram-negative bacilli, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii are particularly important because they frequently acquire extended-spectrum beta-lactamases, carbapenemases, aminoglycoside-modifying enzymes, and fluoroquinolone resistance determinants. ESBL-producing Enterobacterales compromise the activity of third-generation cephalosporins, while carbapenem-resistant Gram-negative bacilli create serious therapeutic limitations [11-13].

Gram-positive organisms, especially Staphylococcus aureus and Enterococcus species, also contribute substantially to hospital infection burden. Methicillin-resistant Staphylococcus aureus is endemic in many Indian hospitals and shows variable susceptibility to commonly used anti-staphylococcal agents [14]. Continuous surveillance of MRSA, ESBL production, carbapenem resistance, and overall MDR prevalence is required to support infection prevention strategies, guide empirical prescribing, and reduce unnecessary use of broad-spectrum antibiotics.

The present hospital-based observational study was conducted to assess the pattern of multidrug-resistant bacterial isolates from clinical samples at Maheshwara Medical College, Patancheru, Hyderabad, Telangana, India. The primary objective was to determine the distribution of bacterial isolates obtained from different clinical specimens. The secondary objectives were to estimate the overall MDR prevalence, evaluate organism-wise MDR distribution, describe antibiotic resistance patterns among Gram-negative and Gram-positive isolates, and identify important resistance phenotypes such as ESBL production, carbapenem resistance, and MRSA.

Materials and Methods

Study design and setting: This hospital-based observational study was conducted in the Department of Microbiology, Maheshwara Medical College, Patancheru, Hyderabad, Telangana, India. The study included bacterial isolates recovered from routine clinical specimens received for culture and antimicrobial susceptibility testing. The study period extended from July 2023 to December 2024. The design was selected to describe the local distribution of clinically significant bacterial isolates and their antimicrobial resistance profile at a defined institutional level.

Study population and sample size: A total of 100 non-duplicate bacterial isolates were included. Isolates were obtained from urine, pus/wound swabs, sputum or other respiratory samples, blood, sterile body fluids, and catheter-related samples. Only one isolate per patient per infection episode was considered to avoid duplication. Culture-positive samples yielding clinically significant bacterial growth were included. Contaminants, repeat isolates from the same patient, mixed growth without clear clinical significance, fungal isolates, and incomplete laboratory records were excluded from analysis.

Sample processing and bacterial identification: Clinical samples were processed according to routine bacteriological procedures. Specimens were inoculated on appropriate culture media and incubated under standard aerobic conditions. Colony morphology, Gram staining, and conventional biochemical reactions were used for organism identification. Isolates were grouped as Gram-negative and Gram-positive bacteria. Enterobacterales, non-fermenting Gram-negative bacilli, staphylococci, and enterococci were reported according to standard laboratory identification practices.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following standardized principles for disc diffusion testing [5]. Antibiotic panels were selected according to organism group and routine laboratory policy. For Gram-negative isolates, the tested drugs included beta-lactams, cephalosporins, fluoroquinolones, aminoglycosides, cotrimoxazole, piperacillin-tazobactam, carbapenems, and colistin where applicable. For Gram-positive isolates, penicillin, erythromycin, ciprofloxacin, clindamycin, gentamicin, methicillin/cefoxitin screening, vancomycin, and linezolid were considered. ESBL production was assessed among Enterobacterales using phenotypic screening and confirmatory principles described for ESBL detection [6]. Methicillin resistance among Staphylococcus aureus isolates was interpreted using cefoxitin/methicillin resistance screening.

Operational definitions and statistical analysis: Multidrug resistance was defined as resistance to at least one antimicrobial agent in three or more antimicrobial categories, consistent with the accepted international definition [4]. ESBL-producing Enterobacterales, carbapenem-resistant Gram-negative bacilli, and MRSA were recorded as important resistance phenotypes. Data were entered into a spreadsheet and analysed descriptively. Categorical variables were expressed as number and percentage. Organism distribution, specimen-wise resistance, and antibiotic-wise resistance patterns were presented in tabular form.

Ethical considerations: The analysis was based on routine diagnostic microbiology records and anonymized isolate-level data. No direct patient contact, additional intervention, or modification of treatment was involved. Patient identifiers were removed before data compilation, and confidentiality was maintained throughout the study. Institutionazzl permission and ethical clearance were considered according to local institutional requirements for record-based observational research.

Results

A total of 100 non-duplicate bacterial isolates obtained from various clinical samples were included in the study. Gram-negative organisms were predominant, accounting for 76.0% of isolates, while Gram-positive organisms constituted 24.0%. Urine was the most common clinical sample, followed by pus/wound swabs, respiratory samples, blood, and other samples. Overall, multidrug resistance was observed in 64 isolates, giving an MDR prevalence of 64.0%. The distribution of clinical samples and MDR isolates is shown in Table 1.

Table 1. Distribution of clinical samples and MDR isolates

Clinical sample	Total isolates	Percentage	MDR isolates	MDR percentage
Urine	38	38.0	28	73.7
Pus/wound swab	24	24.0	16	66.7
Sputum/respiratory sample	16	16.0	10	62.5
Blood	14	14.0	6	42.9
Body fluids/catheter-related samples	8	8.0	4	50.0
Total	100	100.0	64	64.0

Escherichia coli was the most frequently isolated organism, followed by Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Acinetobacter baumannii. The highest MDR proportion was observed among Acinetobacter baumannii isolates, followed by Klebsiella pneumoniae and Escherichia coli. The organism-wise distribution and MDR pattern are presented in Table 2.

Table 2. Organism-wise distribution and multidrug resistance pattern

Bacterial isolate	Total isolates	Percentage	MDR isolates	MDR percentage
Escherichia coli	30	30.0	22	73.3
Klebsiella pneumoniae	20	20.0	15	75.0
Pseudomonas aeruginosa	12	12.0	7	58.3
Acinetobacter baumannii	8	8.0	7	87.5
Proteus mirabilis	4	4.0	2	50.0
Enterobacter species	2	2.0	1	50.0
Staphylococcus aureus	12	12.0	6	50.0
Enterococcus species	7	7.0	3	42.9
Coagulase-negative Staphylococci	5	5.0	1	20.0
Total	100	100.0	64	64.0

Among Gram-negative isolates, high resistance was observed against ampicillin/amoxicillin-clavulanate, ceftriaxone, ceftazidime, and ciprofloxacin. Comparatively lower resistance was noted against amikacin and meropenem. Colistin showed the lowest resistance among the tested antibiotics. The antibiotic resistance pattern among Gram-negative isolates is shown in Table 3.

Table 3. Antibiotic resistance pattern among Gram-negative isolates [n=76]

Antibiotic tested	Resistant isolates	Resistance percentage
Ampicillin/amoxicillin-clavulanate	66	86.8
Ceftriaxone	58	76.3
Ceftazidime	54	71.1
Ciprofloxacin	50	65.8
Cotrimoxazole	44	57.9
Gentamicin	38	50.0
Piperacillin-tazobactam	31	40.8
Amikacin	24	31.6
Meropenem	16	21.1
Colistin	2	2.6

Among Gram-positive isolates, higher resistance was observed against penicillin, erythromycin, and ciprofloxacin. Methicillin resistance was detected in 5 out of 12 Staphylococcus aureus isolates, indicating an MRSA rate of 41.7%. All Gram-positive isolates were sensitive to vancomycin and linezolid. ESBL production was detected in 35 of 56 Enterobacterales isolates, while carbapenem resistance was observed in 16 of 76 Gram-negative isolates. The resistance pattern among Gram-positive isolates and key resistance phenotypes is summarized in Table 4.

Table 4. Resistance pattern among Gram-positive isolates and important resistance phenotypes

Parameter	Denominator	Resistant/positive isolates	Percentage
Penicillin resistance	24	20	83.3
Erythromycin resistance	24	16	66.7
Ciprofloxacin resistance	24	14	58.3
Clindamycin resistance	24	10	41.7
Gentamicin resistance	24	8	33.3
Methicillin resistance among staphylococci	17	7	41.2
MRSA among Staphylococcus aureus	12	5	41.7
Vancomycin resistance	24	0	0.0
Linezolid resistance	24	0	0.0
ESBL-producing Enterobacterales	56	35	62.5
Carbapenem-resistant Gram-negative bacilli	76	16	21.1
Overall MDR among all isolates	100	64	64.0

The findings show a high burden of multidrug-resistant bacterial isolates in the hospital setting. MDR was mainly observed among Gram-negative bacilli, especially Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii. Urine and pus/wound samples contributed the largest proportion of MDR isolates, indicating the need for regular antimicrobial susceptibility surveillance and rational antibiotic use.

Discussion

The present study demonstrated a high burden of multidrug resistance among bacterial isolates obtained from routine clinical samples, with an overall MDR prevalence of 64.0%. This finding reflects the growing challenge of antimicrobial resistance in hospital settings, where repeated antibiotic exposure, severe illness, invasive procedures, and cross-transmission contribute to selection and spread of resistant pathogens. The predominance of Gram-negative organisms in this study is consistent with previous surveillance reports showing that Gram-negative bacilli are major contributors to hospital-acquired and healthcare-associated infections in resource-limited settings [3,7,8].

Urine was the most common clinical sample and also contributed the largest number of MDR isolates. This pattern is clinically important because urinary tract infections are frequently treated empirically, and resistance among uropathogens directly affects initial antibiotic choice. Escherichia coli was the leading isolate, followed by Klebsiella pneumoniae. Similar trends have been reported in hospital-based bacterial surveillance studies, where Enterobacterales commonly predominate among urine, blood, and wound isolates [7,9,10]. The high MDR proportions among Escherichia coli and Klebsiella pneumoniae in the present study indicate that routine empirical use of third-generation cephalosporins and fluoroquinolones requires careful review.

Acinetobacter baumannii showed the highest organism-specific MDR proportion in this study. Although the number of Acinetobacter isolates was small, the high resistance proportion is clinically relevant because this organism is strongly associated with hospital environments, device-associated infections, and limited therapeutic options. Pseudomonas aeruginosa also showed notable MDR prevalence. These non-fermenting Gram-negative bacilli are known for intrinsic resistance mechanisms, biofilm formation, efflux pumps, and acquisition of carbapenemase genes [13]. Lower resistance to meropenem and colistin compared with cephalosporins and fluoroquinolones suggests that reserve antibiotics retain activity in some isolates, but their use demands stewardship oversight.

The resistance profile among Gram-negative isolates showed high resistance to ampicillin/amoxicillin-clavulanate, ceftriaxone, ceftazidime, and ciprofloxacin. This pattern corresponds with the observed ESBL rate of 62.5% among Enterobacterales. ESBL-producing organisms are clinically important because they hydrolyse expanded-spectrum cephalosporins and often carry additional resistance determinants, leading to co-resistance to aminoglycosides and fluoroquinolones [11,12]. Carbapenem resistance among 21.1% of Gram-negative bacilli is also significant, as carbapenem-resistant Enterobacterales and non-fermenters are associated with higher morbidity, treatment failure, and infection-control concern [13].

Among Gram-positive isolates, penicillin, erythromycin, and ciprofloxacin resistance were common, while vancomycin and linezolid resistance were not detected. The MRSA proportion of 41.7% among Staphylococcus aureus isolates is comparable with Indian surveillance data showing substantial methicillin resistance in tertiary-care settings [14]. These findings support the need for periodic local antibiogram preparation, restriction of unnecessary broad-spectrum antibiotics, early de-escalation based on culture results, and reinforcement of hand hygiene, environmental cleaning, sample-guided therapy, and antimicrobial stewardship rounds. The data provide a practical baseline for hospital antibiotic policy and future resistance surveillance.

Limitations

The study was hospital-based and included 100 isolates; therefore, the findings represent the local microbiological profile rather than the wider regional pattern. Clinical outcome data, prior antibiotic exposure, ward-wise distribution, and molecular confirmation of resistance genes were not available. Anaerobic organisms, fungal isolates, and repeat isolates were excluded, limiting assessment of mixed infections and longitudinal resistance trends. Interpretation depends on phenotypic susceptibility results and routine laboratory records.

Conclusion

This hospital-based observational study showed that multidrug-resistant bacteria constituted nearly two-thirds of clinical isolates, with Gram-negative bacilli forming the major burden. Escherichia coli and Klebsiella pneumoniae were the most frequent MDR organisms, while Acinetobacter baumannii showed the highest organism-specific MDR proportion. High resistance to cephalosporins and fluoroquinolones, along with notable ESBL and carbapenem resistance rates, indicates restricted empirical treatment options. MRSA also remained an important Gram-positive resistance phenotype. Routine culture-based diagnosis, periodic antibiogram generation, antimicrobial stewardship, and strict infection-control measures are essential to guide therapy, preserve reserve antibiotics, and reduce further spread of resistant organisms in the hospital setting locally over time.

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