ORIGINAL ARTICLE www.pimr.org.in

DOI: 10.47799/pimr.1401.26.2

Multidrug-Resistant Organisms in Culture-Positive Clinical Samples: Distribution and Clinical Characteristics from a Tertiary Care Hospital in South India

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Date of Submission: 27-02-2026 **Date of Review: 11-03-2026** **Date of Acceptance: 13-04-2026**

ABSTRACT

Background: Multidrug-resistant organisms (MDROs) are a growing problem in hospital practice, limiting treatment options and complicating patient care. Local data on their distribution and associated clinical features are important for guiding infection control and antimicrobial use, particularly in tertiary care settings. **Methods:** A retrospective descriptive study was conducted in a tertiary care teaching hospital in South India. Microbiology records of all culture-positive clinical samples from hospitalized patients over six months were reviewed. Only the first isolate per patient was included. MDROs were defined as extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, carbapenem-resistant Enterobacterales (CRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus* (VRE). Clinical data were obtained for MDRO-positive patients and analyzed descriptively. **Results:** Of 982 samples processed, 300 were culture-positive. Among these, 96 were MDROs, giving a proportion of 32.0% (95% CI: 26.7–37.6). Gram-negative bacilli accounted for 84.4% of MDROs. ESBL-producing Enterobacterales (46.9%) and CRE (40.6%) predominated, while MRSA (12.5%) and VRE (3.1%) were less frequent. *Escherichia coli* and *Klebsiella pneumoniae* were the most common species. Mean patient age was 58.3 ± 16.2 years, and nearly two-thirds were male. Recent antimicrobial use, prior hospitalization, and indwelling devices were frequently observed. Urine was the most common specimen source, with ESBL organisms predominating, while blood cultures more often yielded CRE and MRSA. **Conclusions:** MDROs accounted for a substantial proportion of culture-positive isolates, with Gram-negative organisms predominating. The frequent occurrence of recent antimicrobial exposure, prior hospitalization, and device use highlights the need for closer attention to antimicrobial prescribing and infection control in similar hospital settings.

KEYWORDS: MDRO, ESBL, CRE, MRSA, VRE, Healthcare-associated infections, Antimicrobial resistance

INTRODUCTION

Healthcare-associated infections (HAIs) remain an important cause of morbidity, mortality, and increased healthcare costs in hospitalized patients[1]. The rise of multidrug-resistant organisms (MDROs) has made treatment more difficult and is associated with poorer clinical outcomes[2, 3]. MDROs are defined as organisms that are non-susceptible to multiple classes of antimicrobial agents and grouped as multidrug-resistant (MDR), extensively drug-resistant (XDR)[4].

Important organisms include extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacilli, carbapenem-resistant Enterobacterales (CRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus* spp. (VRE), all of which are commonly found in hospital settings and are associated with adverse clinical outcomes[5-7, 17, 18]. In India, many factors including widespread antibiotic use, environmental dissemination, and healthcare-associated transmission are responsible for antimicrobial resistance [8, 9]. Studies from different regions of India have reported a high burden of resistant Gram-negative organisms, particularly ESBL producers and carbapenem-resistant isolates[10, 11]. Similar findings have been reported from

other regions, where MDRO proportions among clinical isolates range between 30% and 40%[12].

In addition to microbiological patterns, patient-related factors such as prior antimicrobial exposure, hospitalization, invasive devices, and comorbid conditions have been linked to MDRO infections in hospital settings[13-15]. The growing challenge of antimicrobial resistance has been widely recognized as a threat to future therapeutic options[3, 17, 18].

Local data on MDRO distribution and patient characteristics are needed to guide empirical therapy, inform infection prevention practices, and support antimicrobial stewardship efforts at the institutional level.

The present study describes the proportion and distribution of MDROs among culture-positive clinical samples, along with the demographic and clinical profile of hospitalized patients with MDRO infections, in a tertiary care teaching hospital in South India.

METHODS

Study design and setting

A laboratory-based retrospective descriptive study was conducted in the Department of Microbiology at Apollo Institute of Medical Sciences and Research, a tertiary care teaching hospital in Hyderabad, Telangana, India from 1 July to 31 December 2022.

The study was approved by the Institutional Ethics Committee of Apollo Institute of Medical Sciences and Research (EC/NEW/INST/1527/2023/04/054, dated 17-06-2023). As this was a retrospective study using de-identified data, a waiver of informed consent was granted.

Study population and data inclusion

Microbiology laboratory records of all culture-positive clinical samples from hospitalized patients during the study period were retrospectively reviewed, and relevant data were extracted.

To avoid duplication, only the first culture-positive isolate per patient was included in the analysis and repeat isolates from the same patient were excluded. No sampling was performed, and all available records meeting these criteria during the study period were included.

Laboratory procedures

All microbiological investigations were performed as part of routine clinical care in the Department of Microbiology by trained microbiology personnel following standard laboratory protocols. The corresponding data were retrospectively retrieved from the laboratory information system for study purpose.

Clinical specimens, including urine, blood, respiratory samples, wound/pus, and body fluids, were processed according to standard microbiological procedures. Samples were cultured on appropriate media (blood agar, MacConkey agar, and chocolate agar), and isolates were identified using conventional biochemical methods and/or the Vitek 2 automated identification system (bioMérieux, Marcy-l'Étoile, France).

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines (Performance Standards for Antimicrobial Susceptibility Testing), available at the time of testing.

Quality control procedures were carried out following CLSI recommendations using reference strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853). These strains were tested with each new batch of media and antimicrobial discs and at least weekly thereafter. Results were considered valid only if quality control values fell within CLSI-specified acceptable ranges. The same strains were also used as negative controls during phenotypic screening and confirmatory testing.

Extended-spectrum β-lactamase (ESBL)-producing Enterobacterales were identified using standard screening methods and confirmed by the combination disk method (cephalosporin with and without clavulanic acid), with an increase in inhibition zone diameter of ≥5 mm in the presence of clavulanate indicating ESBL production.

Carbapenem-resistant Enterobacterales (CRE) were defined as isolates showing non-susceptibility to at least one carbapenem (imipenem, meropenem, or ertapenem) according to CLSI breakpoints. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) were identified using disk diffusion methods and interpreted according to CLSI criteria.

All isolates demonstrating phenotypic resistance were further tested using the Vitek 2 automated system (AST-GN and AST-GP cards, bioMérieux) to confirm antimicrobial susceptibility profiles.

Definition of multidrug-resistant organisms

Multidrug resistance was defined according to the criteria proposed by Magiorakos *et al*., as non-susceptibility to at least one agent in three or more antimicrobial categories. In this study, a restricted operational definition was applied, and only ESBL-producing Enterobacterales, CRE,

MRSA, and VRE were classified as multidrug-resistant organisms (MDROs).

If an Enterobacterales isolate exhibited both ESBL production and carbapenem resistance, it was counted once in the overall MDRO proportion but included in subtype-specific analyses. Other multidrug-resistant organisms (e.g., *Pseudomonas aeruginosa* and *Acinetobacter baumannii* without carbapenem resistance) were excluded due to inconsistent availability of standardized classification data and the study's focus on priority sentinel MDROs.

Data collection

Clinical and demographic data were retrospectively extracted from patient case records for patients with MDRO-positive isolates.

The variables collected included age, sex, hospital location (ICU, general ward, emergency), prior hospitalization within the preceding 12 months, recent surgery (≤30 days), presence of invasive devices, antimicrobial exposure within the preceding 90 days, duration of hospital stay prior to culture positivity, comorbidities (diabetes mellitus, chronic kidney disease, malignancy, immunosuppression), and specimen source.

Statistical analysis

Data were analyzed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The proportion of multidrug-resistant organisms (MDROs) was calculated as the number of MDRO isolates divided by the total number of culture-positive isolates. Ninety-five percent confidence intervals (95% CIs) were calculated using the binomial exact (Clopper–Pearson) method. Descriptive statistics were used to summarize variables, expressed as frequencies, percentages, and means or medians as appropriate. No inferential statistical comparisons were performed, as clinical and risk-factor data were not available for non-MDRO patients.

Handling of missing data

As this was a retrospective study based on routinely collected clinical records, some variables had missing values. Analyses were conducted using a complete-case approach, with denominators adjusted for each variable as appropriate. No imputation of missing data was performed.

RESULTS

During the study period, 982 clinical samples were processed, of which 300 were culture-positive isolates (30.5%). Among these, 96 isolates were identified as multidrug-resistant organisms (MDROs), corresponding to a proportion of 32.0% (96/300; 95% CI: 26.7–37.6). The remaining isolates were non-MDROs.

Demographic and Clinical characteristics of patients with MDRO isolates

Among patients with MDRO-positive isolates (n = 96), the mean age was 58.3 ± 16.2 years (median 61; range 18–92), with the majority aged 50 years or older. Males accounted for nearly two-thirds of cases. At the time of culture, about half of the patients were admitted to general wards, while the remainder were managed in the intensive care unit or emergency department [Table. 1].

Characteristics	No. (%)
Age (years)	
18–29	6 (6.3)
30–49	18 (18.8)
50–69	46 (47.9)
≥70	26 (27.1)
Sex	
Male	62 (64.6)
Female	34 (35.4)
Ward location	
ICU	28 (29.2)
General ward	48 (50.0)
Emergency	20 (20.8)

Table 1: Demographic characteristics of patients with MDRO isolates (n = 96)

Additional information: Mean age 58.3 ± 16.2 years; median 61 (range 18–92).

Recent healthcare exposure was common in this group. Three-quarters of patients had received antimicrobial therapy within the preceding 90 days, and nearly two-thirds had a history of hospitalization in the past year. Indwelling devices were present in over half of the patients, most frequently urinary catheters, followed by central venous access and mechanical ventilation. Recent surgical procedures were noted in over one-third, and a similar proportion required ICU care. Comorbid conditions were documented in 91%, with hypertension and diabetes mellitus being the most frequent. Overall, most patients had at least one documented healthcare exposure or underlying comorbidity [Table. 2]. The mean duration of hospital stay prior to culture positivity was 9.8 ± 7.2 days.

Microbiological characteristics of MDRO isolates

Gram-negative bacilli were identified in majority of MDROs (84.4%, 81/96), while remaining were

Risk Factors	No. (%)
Prior antimicrobial exposure (≤90 days)	72 (75.0)
Prior hospitalization (≤12 months)	61 (63.5)
Indwelling device present	52 (54.2)
Recent surgery (≤30 days)	34 (35.4)
ICU admission	28 (29.2)
Any comorbidity	71 (74.0)
≥1 exposure/comorbidity	88 (91.7)
None documented	8 (8.3)

Table 2: Healthcare exposures and comorbidities among patients with MDRO isolates (n = 96)

Gram-positive cocci. ESBL-producing Enterobacterales were the most frequently identified MDRO category, followed closely by carbapenem-resistant Enterobacterales (CRE), whereas MRSA and VRE identified in few isolates [Table. 3]. A small number of isolates exhibited overlapping ESBL and carbapenem-resistant phenotypes and were counted once in the overall MDRO total.

MDRO Categories	No. (%)
ESBL-producing Enterobacterales	45 (46.9)
Carbapenem-resistant Enterobacterales (CRE)	39 (40.6)
MRSA	12 (12.5)
VRE	3 (3.1)

Table 3: Distribution of multidrug-resistant organism (MDRO) categories (n = 96)

Note: Three Enterobacterales isolates exhibited both ESBL production and carbapenem resistance and are included in both categories but counted once in the total.

At the species level, *Escherichia coli* and *Klebsiella pneumoniae* predominated among both ESBL- and CRE-producing isolates, with other Enterobacterales and Gram-positive organisms were accounted in smaller numbers [Table. 4].

Urine was the most common source of MDRO isolates, followed by blood, respiratory specimens, wound or pus samples, and body fluids. The distribution of MDRO categories varied across specimen types [Table. 5].

In urine samples (n = 38), ESBL-producing Enterobacterales were the most frequently identified isolates, accounting for just over half of MDROs, followed by CRE and a small number of VRE. In contrast, blood cultures (n = 22) were largely composed of CRE and MRSA, with relatively fewer ESBL-producing organisms. Respiratory specimens (n = 18) were predominantly had CRE, while wound and pus samples (n = 12) showed a more even distribution, with ESBL, CRE, and MRSA all present in comparable proportions.

Organism	Phenotype	No. (%)
Escherichia coli	ESBL	24 (25.0)
Klebsiella pneumoniae	ESBL	16 (16.7)
Enterobacter cloacae complex	ESBL	3 (3.1)
Citrobacter freundii	ESBL	2 (2.1)
Klebsiella pneumoniae	CRE	20 (20.8)
Escherichia coli	CRE	11 (11.5)
Enterobacter cloacae complex	CRE	4 (4.2)
Proteus vulgaris	CRE	2 (2.1)
Citrobacter koseri	CRE	2 (2.1)
Escherichia coli	ESBL + CRE	1 (1.0)
Klebsiella pneumoniae	ESBL + CRE	2 (2.1)
Staphylococcus aureus	MRSA	12 (12.5)
Enterococcus faecalis	VRE	1 (1.0)
Enterococcus faecium	VRE	2 (2.1)

Table 4: Species and phenotype distribution of MDROs (n = 96)

Note: Percentages are calculated using total MDRO isolates (n = 96).

Specimen	ESBL (%)	n	CRE (%)	n	MRSA (%)	n	VRE (%)	n
Urine (n=38)	21 (55.3)		15 (39.5)		–		2 (5.3)	
Blood (n=22)	3 (13.6)		10 (45.5)		9 (40.9)		–	
Respiratory (n=18)	7 (38.9)		11 (61.1)		–		–	
Wound/Pus (n=12)	5 (41.7)		4 (33.3)		3 (25.0)		–	
Body fluids (n=6)	3 (50.0)		3 (50.0)		–		–	

Table 5: Specimen-wise distribution of MDRO categories (n = 96)

Note: Percentages are calculated within each specimen type.

DISCUSSION

Multidrug-resistant organisms are a serious problem in hospital practice, particularly in tertiary care settings where antibiotic exposure and invasive procedures are common. MDROs accounted for 32.0% of culture-positive isolates, comparable to MDRO rates around 30–40% reported from similar settings in India and other regions[10-12]. 84.4% of them were Gram-negative

organisms, similar to pattern reported in hospital-based studies where Enterobacterales and other Gram-negative pathogens make up most resistant isolates[10, 11, 16].

ESBL-producing Enterobacterales and CRE were more common MDROs in this study. Studies from India and neighbouring regions have reported similar findings, with frequent ESBL production and carbapenem resistance, particularly in *Escherichia coli* and *Klebsiella pneumoniae*[7, 10, 11]. Basak *et al*. reported a high proportion of ESBL-producing isolates, while Deka et al. described a predominance of resistant Gram-negative organisms in surgical site infections[10, 11]. Gram-positive MDROs were less frequent, with MRSA more common than VRE. VRE remains uncommon in general hospital populations, although higher rates of colonization have been described in ICU and haemodialysis patients[5, 6].

The distribution of MDROs also varied across specimen types. Urine was the most frequent source and was largely composed of ESBL-producing Enterobacterales, similar to reports identifying *E. coli* and *Klebsiella* spp. as common resistant uropathogen[10, 11]. Blood and respiratory samples showed a higher proportion of CRE and MRSA, similar to studies reporting in hospital-acquired infections and in critically ill patients[11, 12, 15]. These differences may be related to the site of infection, patient characteristics and the level of healthcare exposure.

Majority of patients with MDRO-positive isolates at least one recent healthcare exposure or underlying comorbidities. Prior antimicrobial use, recent hospitalization, invasive device use, and ICU admission were commonly recorded. Studies such as that by Alsehemi et al. have identified similar factors as contributors to MDRO infections in hospitalized patients[13-15]. As a descriptive study, independent risk factors were not assessed; however, the findings outline the clinical profile of patients in whom MDROs were identified.

The high proportion of ESBL-producing organisms, along with the presence of CRE, has implications for empirical therapy. The high proportion of ESBL-producing organisms suggests limited utility of third-generation cephalosporins in serious infections in this setting, and carbapenem resistance further limits treatment options[3]. Timely microbiological diagnosis, selection of empirical therapy based on local data, and subsequent adjustment according to susceptibility results are important for better management. Strengthening antimicrobial stewardship and infection prevention practices remains essential in addressing the burden of MDROs[8, 9].

Overall, these findings reflect the continued predominance of Gram-negative resistance in hospital settings and the close association of MDRO infections with prior healthcare exposure. The study provides local data that may help guide empirical treatment decisions and support ongoing infection control and stewardship efforts in similar settings[17, 18].

Limitations

This was a single-center retrospective study, which may limit generalizability and depended on the completeness of available records. Clinical data were collected only for MDRO-positive patients, so comparisons with non-MDRO infections and assessment of independent risk factors were not possible. Outcomes such as treatment response and mortality were also not evaluated.

CONCLUSION

MDROs were common among culture-positive isolates in this setting, with most isolates being Gram-negative organisms including ESBL-producing Enterobacterales and CRE in majority of cases. Urine was the most common specimen source, with ESBL-producing organisms seen most often, whereas blood and respiratory specimens more frequently had CRE and MRSA.

Recent healthcare exposure and comorbidities were frequently observed among affected patients. These findings reflect the ongoing burden of Gram-negative resistance in routine hospital practice and support the use of local data to guide empirical therapy, along with continued emphasis on antimicrobial stewardship and infection control.

DISCLOSURE

Conflicts of Interest: There are no conflicts of interest to declare.

Funding: No external funding was received for this study.

Ethical Approval: Obtained from Institutional Ethics Committee, Apollo Institute of Medical Sciences and Research (IEC Reference: EC/NEW/INST/1527/2023/04/054, dated 17-06-2023).

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How to cite this article: Goka S. Multidrug-Resistant Organisms in Culture-Positive Clinical Samples: Distribution and Clinical Characteristics from a Tertiary Care Hospital in South India. Perspectives in Medical Research 2026; 14(1):40-45 DOI: 10.47799/pimr.1401.26.2