Section: Community Medicine

Original Research Article

ANTIBIOTIC RESISTANCE TRENDS AND MULTIDRUG RESISTANCE PATTERNS IN CLINICAL ISOLATES AT A TERTIARY CARE HOSPITAL IN DHULE, MAHARASHTRA, INDIA: A RETROSPECTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a growing global threat, particularly in healthcare settings where multidrug-resistant (MDR) clinical isolates complicate treatment outcomes. This study aimed to determine the distribution of clinical isolates, their antimicrobial resistance profiles, and MDR prevalence in a tertiary care hospital in Dhule, Maharashtra, India.

Materials and Methods: A retrospective cross-sectional study was conducted on 402 clinical specimens collected between March 2025 and June 2025. Culture and sensitivity testing were performed, and AMR was interpreted according to CLSI 2024 guidelines. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Contaminated samples were excluded from resistance analysis. Data were analysed using descriptive statistics and Chi-square tests.

Results: Of 402 samples, 357 yielded growths, identifying 27 categories, including an aggregated 'Others' group. Gram-negative predominated, with Escherichia coli (28.29%) and Klebsiella pneumoniae (24.37%) most common; Gram-positive were led by Staphylococcus aureus (14.29%) and MRSA (6.72%). E. coli showed highest resistance to ciprofloxacin (72.58%), while K. pneumoniae was most resistant to cefepime (74.00%). MRSA showed 97.30% resistance to benzylpenicillin. Overall MDR prevalence was 81.89%, highest in orthopedics (87.50%) and ICU (70.73%), and elevated in 46–55 years (79.41%), the largest group. Contamination occurred in 11.19% of samples, chiefly due to improper collection.

Conclusion: High MDR prevalence, particularly among key clinical isolates, highlights the urgent need for targeted antimicrobial stewardship and improved infection control. Regular local antibiogram updates and training to reduce contamination rates are essential.

Keywords: Antimicrobial resistance, multidrug resistance, Escherichia coli, Klebsiella pneumoniae, MRSA, Maharashtra.

INTRODUCTION

Antimicrobial resistance (AMR) occurs when microorganisms evolve to resist medications that once effectively treated them, leading to harder-to-treat infections, prolonged illness, and increased mortality and morbidity.[1] In 2019, AMR was directly responsible for approximately 1.27 million deaths globally and contributed to 4.95 million overall, making it one of the leading causes of

avoidable deaths.[2] Projections by the Wellcome Trust and the GRAM Project suggest that in South Asia, AMR could cause up to 1.91 million annual deaths by 2050, with 8.22 million deaths associated with resistant infections.[3] In India, surveillance data from ICMR's NARS-Net network reveal that more than 70% of E. coli isolates are extended-spectrum β-lactamase (ESBL) producers, while carbapenem resistance has reached about 35% in E. coli and 47% in K. pneumoniae (blood isolates) as of 2022.[4] Multidrug-resistant (MDR) bacteria—defined as resistant to at least one agent in three or more antimicrobial categories—carry particularly high clinical risks.[5] A multi-institutional retrospective study in India demonstrated that patients infected with MDR E. coli, XDR Klebsiella pneumoniae, and MDR Acinetobacter baumannii were two to three times more likely to die compared with those suffering from drug-susceptible infections.[6] International evidence further supports this, with odds ratios for in-hospital mortality ranging from 2.65 to 2.87 for extensively drug-resistant infections.[7] While most Indian AMR data originate from urban tertiary hospitals, emerging evidence from a rural tertiary hospital in Karnataka indicates resistance exceeding 45% to quinolones, penicillins, and cephalosporins in community-acquired pathogens, underscoring the neglected AMR burden in rural areas.[8] To address this gap, the present study aims to create an antibiogram of commonly isolated pathogens, determine the prevalence of MDR organisms, analyze department-wise differences in MDR prevalence, and explore age-related associations. By focusing on a rural tertiary care setting, this research seeks to provide critical local AMR insights, particularly on MDR trends across departments and age groups, which are essential for guiding empirical therapy, strengthening antibiotic stewardship, and shaping public health strategies in rural India.

MATERIALS AND METHODS

This retrospective cross-sectional descriptive-analytical study was conducted at JMF's ACPM Medical College, a tertiary-care teaching hospital in Dhule, Maharashtra, India, with approximately 1,200 beds. Clinical microbiology records and isolates obtained between March and June 2025 were included, while the overall work including literature review, data collection, analysis, and manuscript preparation was carried out between May and August 2025. All culture-positive clinical isolates and samples reported as contaminated during the study period were considered, whereas duplicate isolates from the same patient with identical antibiotic profiles within seven days and samples showing non-significant growth were excluded. Contamination was defined as the recovery of typical skin commensals such as coagulase-negative Staphylococcus spp. from a single culture set without supporting clinical or laboratory evidence of infection, consistent with CDC/NHSN criteria. Specimens had been processed using standard microbiological methods, with identification based on colony morphology, Gram stain, biochemical reactions, and, where required, the VITEK-2 system. Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disk diffusion method and confirmed with VITEK-2 when applicable, with results interpreted according to CLSI 2024 guidelines. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, following standard international definitions.

The required sample size was estimated at 379 using a prevalence of 55.9% MDR reported by Ghosh PK et al., but the actual study population of 402 isolates exceeded this threshold, ensuring adequate power. Data were extracted from laboratory registers and electronic records, anonymized by assigning unique study IDs, and entered into Microsoft Excel 2016. Variables included patient demographics (age, sex, department, diagnosis), sample type (sterile vs. non-sterile), organism isolated, antimicrobial susceptibility profile, and MDR status. Duplicate isolates were removed as per predefined criteria. Statistical analysis comprised descriptive statistics (frequencies and percentages for organism distribution, resistance patterns, and MDR prevalence) and chi-square tests to evaluate associations between MDR status and patient age group or department of admission, with $p < 0.05$ considered statistically significant. Ethical approval was sought from the Institutional Ethics Committee, and the need for informed consent was waived due to the retrospective nature of the study.

RESULTS

A total of 402 clinical samples were collected between March 2025 and June 2025 for culture and sensitivity testing. The most frequent specimen types were Blood (26.61%) & Pus (26.61%) followed by Urine (17.66%), Soft Tissue (14.92%), and others.cOf these, 88.56% were sterile site samples. Contamination was documented in 45 samples (11.19% of all collected), most commonly due to Improper technique of collection, followed by Laboratory or Equipment related errors, Inadequate Sampling Volume, Improper Labelling, and others.

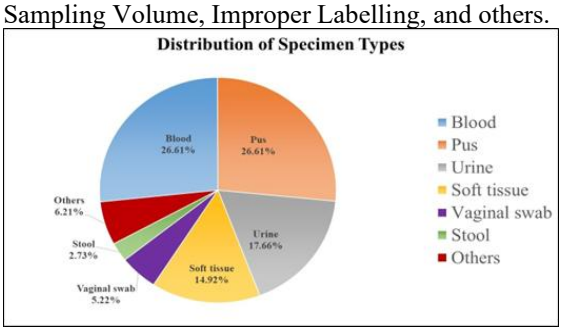

Figure 1: Distribution of specimen types among clinical isolates.

The pie chart illustrates the distribution of specimen types processed in the study. Blood and pus were the most common specimens, each contributing 26.6% of the total. Urine samples accounted for 17.7%, while soft tissue samples comprised 14.9%. Vaginal swabs (5.2%) and stool samples (2.7%) formed smaller proportions, and 6.2% were categorized as other specimen types. This distribution highlights that invasive samples such as blood and pus predominated in microbiological investigations.

The most frequently isolated organism was Escherichia coli (17.16%), followed by Klebsiella pneumoniae (15.42%), Staphylococcus aureus (MRSA) (10.95%), Pseudomonas aeruginosa (7.71%), and others. [Table 1]

Table 1: Distribution of organisms isolated from clinical samples.

Organism isolated from the sample	Number of Isolates	Percentage
Gram Negative Bacteria		
Escherichia coli	69	17.16%
Klebsiella pneumoniae	62	15.42%
Pseudomonas aeruginosa	31	7.71%
Acinetobacter	12	2.99%
Enterobacter cloacae	12	2.99%
Burkholderia cepacia	5	1.24%
Proteus mirabilis	5	1.24%
Morganella morganii	1	0.25%
Proteus vulgaris	1	0.25%
Stenotrophomonas maltophilia	1	0.25%
Gram Positive Bacteria		
Staphylococcus aureus (MRSA)	44	10.95%
Staphylococcus (Coagulase negative)	24	5.97%
Enterococcus faecium	19	4.73%
Staphylococcus aureus	16	3.98%
Staphylococcus haemolyticus	14	3.48%
Staphylococcus hominis	10	2.49%
Streptococcus Group A	10	2.49%
Staphylococcus epidermidis	6	1.49%
Enterococcus gallinarum	3	0.75%
Staphylococcus saprophyticus	3	0.75%
Staphylococcus sciuri	2	0.50%
Other Clinically Significant Isolates		
Candida albicans	10	2.49%
Candida ciferrii	8	1.99%
Candida tropicalis	7	1.74%
Candida guilliermondii	6	1.49%
Trichosporon asahii	1	0.25%
Others	20	4.98%
Grand Total	402	100.00%

When stratified by specimen type, Klebsiella pneumoniae predominated in Blood samples, whereas Staphylococcus aureus (MRSA) was most common in Pus samples.

Among Gram-negative isolates, Escherichia coli was predominant, whereas Staphylococcus aureus (MRSA) was the leading Gram-positive pathogen Only organisms with ≥ 8 isolates were included. MRSA was analysed separately from Staphylococcus aureus due to distinct resistance profiles. Resistance rates calculated on sterile isolates only. While both sterile and contaminated samples were included in the overall analysis, contaminated samples were excluded before calculating antimicrobial resistance rates.

Table 2: Antibiotic Resistance Profile of Gram-Negative Isolates

Antibiotic	Escherichia coli (n=62)	Klebsiella pneumoniae (n=50)	Pseudomonas aeruginosa (n=30)	Acinetobacter (n=12)	Enterobacter cloacae (n=12)
Amikacin	18 (29.03%)	26 (52.00%)	7 (23.33%)	7 (58.33%)	5 (41.67%)
Gentamicin	24 (38.71%)	24 (48.00%)	1 (3.33%)	8 (66.67%)	5 (41.67%)
Tobramycin	1 (1.61%)	7 (14.00%)	0 (0.00%)	3 (25.00%)	0 (0.00%)
Netilmicin	1 (1.61%)	7 (14.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ciprofloxacin	45 (72.58%)	30 (60.00%)	14 (46.67%)	7 (58.33%)	8 (66.67%)
Levofloxacin	6 (9.68%)	8 (16.00%)	12 (40.00%)	5 (41.67%)	1 (8.33%)
Cefuroxime	38 (61.29%)	28 (56.00%)	0 (0.00%)	0 (0.00%)	9 (75.00%)
Cefepime	39 (62.90%)	37 (74.00%)	8 (26.67%)	8 (66.67%)	10 (83.33%)
Ceftriaxone	41 (66.13%)	30 (60.00%)	0 (0.00%)	5 (41.67%)	10 (83.33%)
Amoxicillin	38 (61.29%)	28 (56.00%)	0 (0.00%)	0 (0.00%)	11 (91.67%)
Meropenem	22 (35.48%)	30 (60.00%)	9 (30.00%)	6 (50.00%)	7 (58.33%)
Polymyxin B	1 (1.61%)	2 (4.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Trimethoprim/ Sulfamethoxazole	30 (48.39%)	19 (38.00%)	1 (3.33%)	7 (58.33%)	4 (33.33%)
Nitrofurantoin	3 (4.84%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

Among Gram-negative isolates, high levels of resistance were observed to fluoroquinolones and cephalosporins. Escherichia coli showed 72.6% resistance to ciprofloxacin and over 60% resistance to cefuroxime, cefepime, ceftriaxone, and amoxicillin. Klebsiella pneumoniae also exhibited high resistance, with 60% resistant to ciprofloxacin and meropenem, and 74% to cefepime. Pseudomonas aeruginosa demonstrated comparatively lower resistance, particularly to aminoglycosides, but still showed 46.7% resistance to ciprofloxacin. Acinetobacter isolates were highly resistant, with 58.3% resistant to amikacin, ciprofloxacin, and trimethoprim-sulfamethoxazole, and 50% to meropenem. Similarly, Enterobacter cloacae showed very high resistance rates, exceeding 80% for cefepime, ceftriaxone, and amoxicillin. Notably, resistance to polymyxin B remained very low across all species, and nitrofurantoin resistance was largely confined to E. coli.

Table 3: Antibiotic Resistance Profile of Gram-Positive Isolates

Antibiotic	Staphylococcus aureus (MRSA) (n=37)	Staphylococcus (Coagulase negative) (n=24)	Enterococcus faecium (n=16)	Staphylococcus aureus (n=8)	Staphylococcus haemolyticus (n=14)
Benzylpenicillin	36 (97.30%)	22 (91.67%)	10 (62.50%)	7 (87.50%)	14 (100.00%)
Oxacillin	34 (91.89%)	19 (79.17%)	0 (0.00%)	3 (37.50%)	12 (85.71%)
Erythromycin	29 (78.38%)	22 (91.67%)	11 (68.75%)	4 (50.00%)	14 (100.00%)
Clindamycin	26 (70.27%)	19 (79.17%)	0 (0.00%)	5 (62.50%)	12 (85.71%)
Vancomycin	10 (27.03%)	13 (54.17%)	7 (43.75%)	4 (50.00%)	6 (42.86%)
Teicoplanin	4 (10.81%)	13 (54.17%)	3 (18.75%)	0 (0.00%)	6 (42.86%)
Rifampicin	10 (27.03%)	17 (70.83%)	0 (0.00%)	2 (25.00%)	10 (71.43%)
Chloramphenicol	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Tetracycline	5 (13.51%)	10 (70.83%)	13 (81.25%)	0 (0.00%)	3 (21.43%)
Doxycycline	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

Among Gram-positive isolates, very high resistance was observed to benzylpenicillin, with rates exceeding 90% in Staphylococcus aureus (MRSA), coagulase-negative staphylococci, and Staphylococcus haemolyticus. Oxacillin resistance was also marked, particularly in MRSA (91.9%) and S. haemolyticus (85.7%), confirming their multidrug-resistant nature, while Enterococcus faecium remained intrinsically resistant to oxacillin. High levels of resistance to erythromycin and clindamycin were noted across staphylococcal isolates, especially coagulase-negative strains (91.7% and 79.2%, respectively). Alarmingly, resistance to vancomycin was detected, ranging from 27% in MRSA to 54% in coagulase-negative staphylococci and 43.8% in E. faecium. Resistance to teicoplanin followed a similar pattern, with up to 54% in coagulase-negative staphylococci. Rifampicin resistance was notable in S. haemolyticus (71.4%) and coagulase-negative staphylococci (70.8%). In contrast, chloramphenicol and doxycycline showed complete susceptibility across all isolates, while tetracycline resistance was highest in E. faecium (81.3%). These findings underscore the widespread prevalence of multidrug resistance among Gram-positive isolates, with concerning emergence of vancomycin resistance in both staphylococci and enterococci.

Table 4: MDR Prevalence

Organism	Number of Sterile Isolates	Number of MDR samples	MDR Prevalence (%)
Gram Negative Bacteria			
Escherichia coli	62	51	82.26%
Klebsiella pneumoniae	50	39	78.00%
Pseudomonas aeruginosa	30	15	50.00%
Acinetobacter	12	9	75.00%
Enterobacter cloacae	12	12	100.00%
Gram Positive Bacteria			
Staphylococcus aureus (MRSA)	37	37	100.00%
Staphylococcus (Coagulase negative)	24	22	**91.67%**
Enterococcus faecium	16	12	75.00%
Staphylococcus haemolyticus	14	14	100.00%
Staphylococcus aureus	8	6	75.00%
Total/Overall	265	217	81.89%

The prevalence of multidrug resistance (MDR) among clinical isolates was alarmingly high, with an overall MDR rate of 81.9%. Among Gram-negative organisms, Enterobacter cloacae exhibited the highest prevalence with all isolates (100%) classified as MDR, followed by Escherichia coli (82.3%), Klebsiella pneumoniae (78%), and Acinetobacter (75%). Pseudomonas aeruginosa demonstrated relatively lower MDR rates, though still concerning at 50%. Gram-positive organisms showed similarly high resistance patterns, with 100% of Staphylococcus aureus (MRSA) and Staphylococcus haemolyticus isolates being MDR, along with 91.7% of coagulase-negative staphylococci. Enterococcus faecium and methicillin-susceptible Staphylococcus aureus showed MDR prevalence of 75%. These

findings highlight the widespread dominance of MDR pathogens across both Gram-negative and Gram-positive isolates, posing significant therapeutic challenges in the tertiary-care setting.

Table 5: Department-Wise MDR Prevalence

Department	Number of Isolates	Number of MDR Samples	MDR Prevalence (%)	p-Value
ICU	82	58	70.73%	
Orthopedics	72	63	87.50%	
Medicine	67	50	74.63%	
Surgery	65	52	80.00%	
OB/GYN	34	18	52.94%	
Total/Overall	320	241	75.31%	0.0023

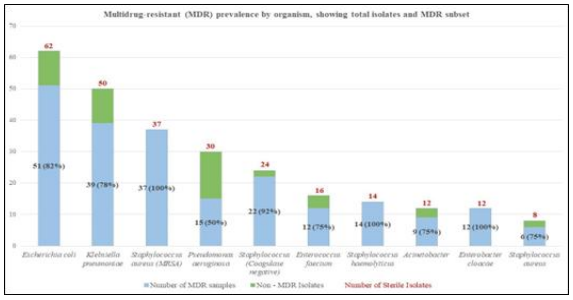

Figure 3: Multidrug-resistant (MDR) prevalence by organism, showing total isolates and MDR subset

The analysis of department-wise MDR prevalence revealed significant variability across clinical specialties, with an overall MDR prevalence of 75.3% (p = 0.0023). The highest prevalence was observed in orthopedic isolates, where 87.5% were MDR, followed by surgery (80.0%) and medicine (74.6%). Intensive Care Unit (ICU) isolates also showed a high MDR burden at 70.7%. In contrast, isolates from obstetrics and gynecology (OB/GYN) demonstrated a comparatively lower prevalence of 52.9%. These findings indicate that while MDR pathogens are widespread across all departments, surgical and orthopedic units are disproportionately affected, possibly reflecting higher exposure to invasive procedures, prolonged hospital stays, and frequent antibiotic use.

Table 6: Age Group-Wise MDR Prevalence*

Age Groups	Number of Isolates	Number of MDR Samples	MDR Prevalence (%)	p-Value
0 - 5 years	8	8	100.00%	
6 - 15 years	6	3	50.00%	
16 - 25 years	18	16	88.89%	
26 - 35 years	53	37	69.81%	
36 - 45 years	89	59	66.29%	
46 - 55 years	102	81	79.41%	
55 - 65 years	68	52	76.47%	
Above 65	13	10	76.92%	
Total/Overall	357	266	74.51%	0.106

The age-wise distribution of MDR prevalence showed consistently high resistance across all age groups, with an overall prevalence of 74.5% (p = 0.106). The highest prevalence was observed in children under 5 years, where all isolates (100%) were MDR, although the sample size was small. Adolescents aged 6–15 years had the lowest prevalence at 50%. Among adults, MDR prevalence ranged between 66.3% and 79.4%, with the highest burden seen in the 46–55 year age group (79.4%), followed by those aged 55–65 years (76.5%) and above 65 years (76.9%). These findings suggest that while MDR pathogens affect all age groups, middle-aged and elderly patients are more vulnerable, likely due to comorbidities, recurrent hospitalizations, and higher cumulative antibiotic exposure.

Table 7: Contamination by Specimen Type

Specimen Type	Number of Samples	Number of Contaminated Samples	Contamination Rate (%)
Blood	107	21	19.63%
Pus	107	10	9.35%
Urine	71	0	0.00%
Soft tissue	60	9	15.00%
Vaginal swab	21	1	4.76%
Stool	11	3	27.27%
Others	25	1	4.00%
Total/Overall	402	45	11.19%

The overall contamination rate among clinical specimens was 11.2%. Blood samples demonstrated a relatively high contamination rate of 19.6%, followed by stool samples at 27.3%, although the latter had a small sample size. Soft tissue specimens also showed considerable contamination (15.0%),

while pus samples had a lower rate at 9.4%. Vaginal swabs and "other" specimen types demonstrated minimal contamination (4.8% and 4.0%, respectively), and no contamination was reported in urine samples. These findings indicate that contamination was most frequent in blood and stool cultures, underscoring the need for strict aseptic collection practices, especially for invasive specimens such as blood.

DISCUSSION

Antimicrobial resistance (AMR) continues to pose a formidable global and national health crisis, and this study reinforces its gravity within our tertiary care centre. The predominant pathogens identified—Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus—are consistent with the most frequently reported organisms in the Indian Council of Medical Research's (ICMR) AMRSN surveillance reports.[12] The high resistance of E. coli to ciprofloxacin and K. pneumoniae to cefepime closely parallels resistance trends documented in multicentric Indian hospital-based surveillance.[13] Similarly, the pronounced resistance of MRSA to benzylpenicillin reflects the escalating national burden of MRSA, where prevalence has risen from approximately 33% to 45% in recent years.[14]

Departmental analysis revealed that the ICU and orthopedics wards harbored the highest MDR prevalence. This finding is in line with previous evidence suggesting that factors such as prolonged hospital stays, use of invasive devices, surgical interventions, and frequent broad-spectrum antibiotic exposure significantly contribute to MDR selection pressure.[12,13] Moreover, the high prevalence of MDR in the 46–55 year age group can be attributed to the larger patient pool with chronic comorbidities, recurrent hospital admissions, and cumulative antibiotic exposure, which collectively increase the risk of colonization and infection by resistant organisms.

Contamination analysis showed an overall rate of ~11%, with the highest rates observed in stool (27.3%) and blood cultures (19.6%). These results highlight persistent challenges in sample collection and processing, where factors such as inadequate aseptic technique, improper specimen handling, insufficient sample volume, and labeling errors play a critical role. Previous studies have emphasized that contamination not only inflates diagnostic costs but also leads to unnecessary antibiotic use and prolonged hospital stay, underscoring the importance of stringent adherence to collection protocols and continuous staff training.[12]

Clinically, the findings underscore limited empirical treatment options for both Gram-negative and Gram-positive infections in our setting. The rising resistance to carbapenems, vancomycin, and other last-resort agents narrows therapeutic choices and increases dependence on toxic or less effective alternatives. This reinforces the urgent need for institutional antimicrobial stewardship programs (ASP), guided by local antibiogram data and harmonized with Clinical and Laboratory Standards Institute (CLSI) recommendations.[10] Targeted stewardship strategies, including restriction of broad-spectrum antibiotics, de-escalation protocols, and continuous monitoring of antibiotic consumption, will be vital in containing resistance.

The strengths of this study include its adequate sample size, stratified analysis across departments and age groups, and alignment with national AMR surveillance frameworks. However, limitations must be acknowledged. Being a single-center study conducted over a limited time frame, the results may not fully capture seasonal or regional variations. Molecular mechanisms of resistance were not evaluated, which limits the ability to correlate phenotypic resistance with genetic determinants. Additionally, less common pathogens could not be analyzed due to small sample sizes, which may have introduced bias in overall resistance estimates.

In conclusion, this study demonstrates a high prevalence of MDR pathogens in both Gram-negative and Gram-positive groups, with resistance patterns that closely mirror national surveillance trends. The findings highlight the urgent need for continuous AMR surveillance, evidence-based prescribing, robust antimicrobial stewardship, and reinforced infection prevention and control practices at the institutional level. Without immediate and coordinated action, the therapeutic landscape for common infections will continue to shrink, threatening patient outcomes and increasing healthcare costs.

CONCLUSION

This study demonstrated an alarmingly high overall prevalence of multidrug resistance (MDR) at 81.9% among clinical isolates in our tertiary-care center, with particularly high rates in Enterobacter cloacae (100%), MRSA (100%), and Staphylococcus haemolyticus (100%). Among Gram-negative organisms, Escherichia coli (82.3%) and Klebsiella pneumoniae (78.0%) were the predominant MDR pathogens, while among Gram-positives, coagulase-negative staphylococci exhibited a 91.7% prevalence. Department-wise analysis revealed significantly higher MDR burden in orthopedics (87.5%) and surgery (80.0%) compared to other specialties ($p = 0.0023$), and age-stratified analysis showed maximum MDR prevalence in the 46–55 year age group (79.4%) and universal resistance in isolates from children under five years. Contamination rates averaged 11.2%, with the highest noted in stool (27.3%) and blood (19.6%) specimens. These findings emphasize the critical challenge posed by MDR pathogens in both community- and hospital-acquired infections and highlight the urgent need for strengthened

antimicrobial stewardship, routine local antibiogram updates, stringent infection control measures, and strict adherence to specimen collection protocols.

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