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Original Article

Comprehensive Review of Clinical Isolates and Antibiotic Resistance Trends: A One-Year Laboratory-Based Study

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ABSTRACT

Background: Antimicrobial resistance (AMR) poses a major global health challenge, leading to treatment failure, prolonged hospital stay, and increased mortality. Local hospital antibiograms are essential tools for guiding empirical therapy and strengthening antimicrobial stewardship.
Objectives: To determine the distribution of clinical isolates and assess antibiotic resistance patterns over one year in a tertiary care hospital.
Materials and Methods: This cross-sectional laboratory-based study was conducted from September 2024 to August 2025 at a 1500-bedded tertiary care hospital in Central India. All clinical samples from OPD, IPD, and ICUs were processed using standard microbiological methods. Only the first isolate per patient was included. Identification and antimicrobial susceptibility testing were performed using the VITEK 2 system, interpreted as per CLSI 2024 guidelines, and analyzed using WHONET 2024.
Results: Of 1,065 culture-positive samples, 76% were Gram-negative bacteria, with Escherichia coli being the most common isolate, followed by Klebsiella pneumoniae and Pseudomonas aeruginosa. High resistance was observed among Gram-negative organisms, while fosfomycin and nitrofurantoin remained effective against urinary E. coli. MRSA prevalence was 58%, with linezolid and vancomycin showing high efficacy.
Conclusion: The study demonstrates a high burden of multidrug-resistant pathogens, emphasizing the need for regular antibiogram-based surveillance to support rational antibiotic use.

Keywords: *Antimicrobial Resistance, Antibiogram, Multidrug-Resistant Organisms, Antimicrobial Stewardship, AWaRe Classification.*

INTRODUCTION

Antimicrobial resistance is one of the top ten global public health threats to humanity. It has increased to an alarmingly high level in the past two decades, and the main cause for the same can be safely attributed to irrational usage, overuse and over-the-counter availability of these drugs.[1,2,3]

Infections caused by multi-drug resistant (MDR) pathogens fail to respond to initial or first-line drugs, which results in longer hospital stays and increased mortality rates. Loss of sensitivity to the first line group of antibiotics leads to longer periods of infection with multi-drug resistant organisms and increased numbers of infected people moving in the local population.[4] This, in turn, poses a high risk of transmission of MDR pathogens as well as resistance genes to the general population.

There has been a significant leap and advances in various diagnostic modalities, infection control practices, antimicrobial stewardship programmes, but still; infections with multidrug resistant organisms remains a significant cause of morbidity and mortality among both the in and outpatients of developed as well as developing countries.[5]

The World Health Organization (WHO) has emphasized the key role of the microbiology laboratory and a microbiologist in antimicrobial stewardship (AMS) by guiding clinicians on the appropriate and rational use of antibiotics through the formulation ofantibiograms.[6] Comprehensive data on the antibiotic sensitivity patterns of bacterial pathogens isolated from different samples and wards is needed for compiling an antibiogram for a particular health set-up. The local antibiogram provides a guide to the clinicians and helps them choose the best empirical antimicrobial treatment according to the local antibiogram in the event that microbiology culture and susceptibility results are pending. It also helps in monitoring the trends of resistance to the common antimicrobials within the hospital setup -as well as to compare the susceptibility rates across institutions and track resistance trends. Hence, the local antibiogram from a hospital setting may contribute to the national AMR surveillance database as well.[7]

Antibiogram data must always be formulated from an accredited or certified diagnostic microbiology laboratory that uses standard guidelines like the Clinical Laboratory Standards Institute (CLSI) M39-A4 consensus document[8] to ensure accuracy as well as reliability. As AMR is on the rise, antibiogram serves as a useful weapon to fight this impending doom by providing evidence-based data for prescribing empirical treatment as per the WHO essential Medicines List using the Access, Aware, and Reserve (AWaRe) classification.[9-11] Antimicrobial stewardship program relies solely on the Surveillance data of rising AMR trends, and this can prove to be an effective tool in evidence-based decision making.

Therefore, this study was undertaken to determine the prevalence of microbial isolates and to evaluate the local antibiogram.

MATERIALS AND METHODS

Study Setting: The study was carried out at a 1500 bedded tertiary care Centre and teaching hospital located in Central India. The study was conducted over a 12-month period from September 2024 to August 2025. Samples from OPD, IPD, and ICU were processed in the Department of Microbiology.

Study design: Cross-sectional study
Sample size: All the clinical samples received from OPD/ IPD/ICUs during the specified time period were used for analysis in the study.

Inclusion criteria: Only the first isolate recovered during the specified time period per patient with confirmed identification and susceptibility testing results was included for analysis.

Exclusion Criteria: The following were excluded from the study:

- Isolates of screening or surveillance cultures,
- Isolates with ambiguous or intermediate sensitivity results.,
- Duplicate isolate from the same patient.

Sample processing:

1. Culture & sensitivity: Various clinical samples were categorized into Blood (collected from both central and peripheral lines), Urine (urine collected from Catheterized and Mid-stream urine etc.), Respiratory specimens (Sputum, ET aspirate, BAL, etc.), Exudates (Pus, wound swabs, Tissue etc.), Fluid (CSF, Ascitic fluid, Synovial fluid Etc.). All the samples were processed according to the standard operating procedures of the laboratory.[12,13] Culture-positive samples were further identified and processed for AST by Vitek 2. AST interpretation was done as per CLSI 2024 guidelines.[14]

2. **Data Collection and Analysis:** As this was a retrospective study, hence the data was retrieved through the Hospital Management information system (HMIS) and analyzed and interpreted usingWHO NET 2024 version.

Ethical Approval: As this study was based on secondary data analysis, no ethical approval was needed.

RESULT

Of the total clinical samples processed in the microbiology laboratory, 1,065 samples yielded positive growth. The sample-wise distribution of these positive isolates was as follows: Urine (n = 505, 47%), exudates (n = 183, 17%), Respiratory specimens (n = 182, 17%), Blood (n = 166, 16%), and body fluids (n = 29, 3%). Positive culture yield across different clinical sample types is shown in Chart 1.

Among the culture positive cases, 811 (76%) was attributed to Gram-negative bacteria, and the rest by Gram-positive bacteria, 218 (20%), and fungal 36 (4%) isolates. Organism-wise distribution of the isolates showed *Escherichia coli* [n = 331 (31%)] as the most frequently isolated pathogen, followed by *Klebsiella pneumoniae* [n = 217 (20%)] and *Pseudomonas aeruginosa* [n = 91 (9%)] among the Gram-negative bacteria. The miscellaneous Gram-negative bacilli included *Achromobacter* spp., *Aeromonas* spp., *Proteus* spp., *Providencia* spp., *Morganella* spp., and *Pseudomonas* spp.

other than *P. aeruginosa*. Among the Gram-positive organisms, Enterococcus spp. [n = 98 (9%)] and Staphylococcus aureus [n = 38 (4%)] were the predominant isolates. Various Candida species were also recovered, primarily *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*etc. Notably, the emerging multidrug-resistant Candida auris was also isolated from a few clinical samples. Sample-wise distribution of culture-positive microorganisms is shown in Table 1.

	Table 1:Sample Wise distribution of culture-positive microorganisms						
SN	**Gram Negative Organisms**	**Total**	**Blood**	**Fluid**	**Exudates**	**Respiratory specimens**	**Urine**
1	Acinetobacter baumannii	**51**	5	2	7	31	6
2	Burkholderia cepacia	1	0	0	0	1	0
3	Burkholderia pseudomallei	6	3	0	2	1	0
4	Citrobacter sp.	11	1	0	2	1	7
5	Enterobacter cloacae complex	19	2	0	9	3	5
6	Escherichia coli	**331**	17	7	48	23	236
7	Klebsiella pneumoniae	**217**	32	3	27	64	91
8	pseudomonas aeruginosa	91	3	4	20	24	40
9	Salmonella Typhi	19	18	0	1	0	0
10	Serratia marcescens	14	3	0	0	2	9
11	Stenotrophomonas maltophilia	6	1	0	1	4	0
12	Miscellaneous GNB	45	18	0	7	3	17
13	Enterococcus Spp.	**98**	10	6	12	4	66
14	Staphylococcus aureus	**38**	9	3	19	6	1
15	CONS	77	34	4	24	5	10
16	Streptococcus sp.	5	1	0	2	2	0
17	Candida albicans	5	1	0	1	0	3
18	Candia nonalbicans	31	8	0	1	8	14
	Total	**1065**	**166**	**29**	**183**	**182**	**505**

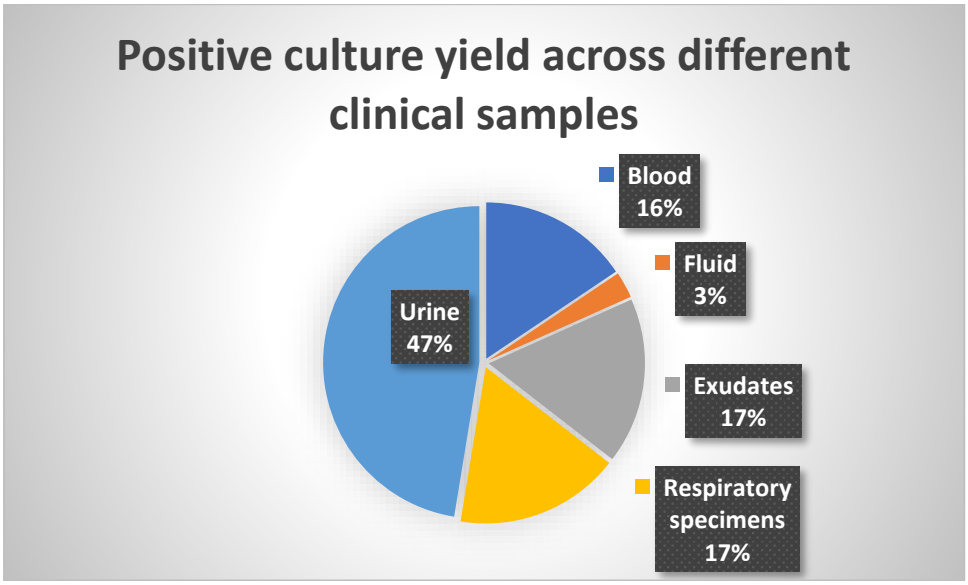

Chart 1: Positive culture yield across different clinical samples

Table 2: Antibiotics susceptibility profile of important Gram Negative Bacilli

Antibiotics		**Gram Negative organism (Total no of isolates)**			
		E. coli (331)	**Klebsiella pneumoniae (217)**	**Pseudomonas aeruginosa (91)**	**Acinetobacter Spp. (51)**
ACCESS	Amoxiclav	34.6	26.9	IR	IR
	Amikcain	70.1	29.2	57.1	11.7
	Gentamcin	65	34.2	40.0	17.6
	Cotrimoxazole	44.2	37.3	IR	38.2
	Doxycycline	37.9	18.6	NR	NR

WATCH	Cefuroxime	11.5	7.6	IR	IR
	Ceftriaxone	22.5	14.1	IR	0
	Ceftazdime	37.5	16.4	58.8	14.2
	Cefepime	25	10.3	57.9	6.8
	Cefoperazone sulbactam	59	18.3	48.8	8.3
	Piperacillin Tazobactam	57.6	20.4	52.3	6.3
	Ampicillin sulbactam	37.5	11	IR	13.6
	Ertapenem	58.8	24.8	IR	IR
	Imipenem	71.1	18	50.5	10.6
	Meropenem	70.8	19.8	52.2	14.2
	Ciprofloxacin	12.1	11.2	46.1	13.7
	Levofloxacin	10.8	8.1	44.7	10
	Ofloxacin	28.5	53.3	NR	0
	Minocycline	68.6	38.6	0	21.9
RESERVE	Aztreonam	40.5	14.2	70.6	IR
	Ceftazidime - Avibactam	78	34	58.1	NR
	cefepime enmetazobactam	33.3	26.6	40	NR
	Colistin	0	0	0	0
URINARY	Fosfomycin	98.2	67	NR	IR
	nitrofurantoin	81.1	11.4	NR	NR
	Norfloxacin	35.6	51.4	35	NR

IR: Intrinsic Resistant, NR: Not reported

Table 3: Antibiotics susceptibility profile of important Gram Positive Cocci

Antibiotics		**Gram positive organism (Total no of isolates)**	
		Enterococcus spp. (98)	**Staphylococcus aureus (38)**
ACCESS	Pencillin	52.5	2.6
	Ampicillin	48.2	NR
	Oxacillin	NR	41.6
	Gentamicin	IR	81
	Clindamycin	NR	45.9
	Cotrimoxazole	IR	67.5
WATCH	Tetracycline	16.6	84.2
	Ciprofloxacin	11.3	5.4
	Levofloxacin	11.8	6.8
	Erythromycin	6.5	31.5
	Minocycline	38.8	NR
	Doxycycline	27.5	NR
	High Level gentamicin	27.9	NR
RESERVE	Linezolid	93.5	100
	Vancomycin	84	94.5
	Teicoplanin	87.3	94.7
URINARY	Nitrofurantoin	55.5	100
	Fosfomycin	7.9	NR
	Norfloxacin	7.5	NR

IR: Intrinsic Resistant, NR: Not reported

Antibiotics were categorized into **Access, Watch, and Reserve (AWaRe)** groups according to the **WHO AWaRe Classification**, which guides optimal antibiotic selection and stewardship. The **Access** group includes first-line agents with lower resistance potential, the **Watch** group comprises broader-spectrum antibiotics with higher resistance risk, and the **Reserve** group contains last-resort agents reserved for treatment of multidrug-resistant infections. Antibiotics with known **intrinsic resistance** for specific organisms were flagged and not interpreted for susceptibility.

Antibiotic susceptibility profile of important Gram-negative bacilli is shown in Table 2. *E. coli* showed good susceptibility to Amikacin (70%), Carbapenems (71% Imipenem, 70.8% Meropenem), Piperacillin–Tazobactam (57.6%),

and Cefoperazone–Sulbactam (59%). Among urinary agents, *E. coli* demonstrated very high susceptibility to Fosfomycin (98.2%) and good response to Nitrofurantoin (81%). *Klebsiella pneumoniae* showed a more resistant pattern than *E. coli*. The majority of the *Klebsiella pneumoniae* isolates were MDRO, isolated from ICU patients. *Klebsiella pneumoniae* showed poor susceptibility to most Access and Watch group antibiotics, with Amikacin (29%) and carbapenems (18–25%) being the only moderately effective agents. *Pseudomonas aeruginosa* retained moderate susceptibility to Ceftazidime (58.8%), Cefepime (57.9%), Piperacillin–Tazobactam (52%), and Carbapenems (~50%). *Acinetobacter* spp. demonstrated high multidrug resistance, with very low susceptibility across all AWaRe categories; Minocycline (21.9%) and Colistin (40%) were the only agents with notable activity. Reserve-group agents like Ceftazidime–Avibactam and Cefepime–Enmetazobactam were tested for MDRO. A double disk synergy test for Ceftazidime Avibactam and Aztreonam was performed, which showed good results with *E. coli*. Fluoroquinolone susceptibility was uniformly poor across all four organisms (≤12% for E. coli; 8–11% for Klebsiella; ~45% for Pseudomonas; 10–13% for Acinetobacter).

Enterococcus spp. showed high susceptibility to Linezolid (93.5%), Vancomycin (84%), and Teicoplanin (87.3%), with moderate response to β-lactams and poor activity of fluoroquinolones. S. aureus demonstrated excellent susceptibility to Linezolid (100%), Vancomycin (94.5%), and Teicoplanin (94.7%), while Gentamicin (81%) and Tetracycline (84.2%) also remained effective. Oxacillin susceptibility was 41.6%, indicating a MRSA prevalence of 58%.

Fluoroquinolone susceptibility was uniformly low in both organisms.

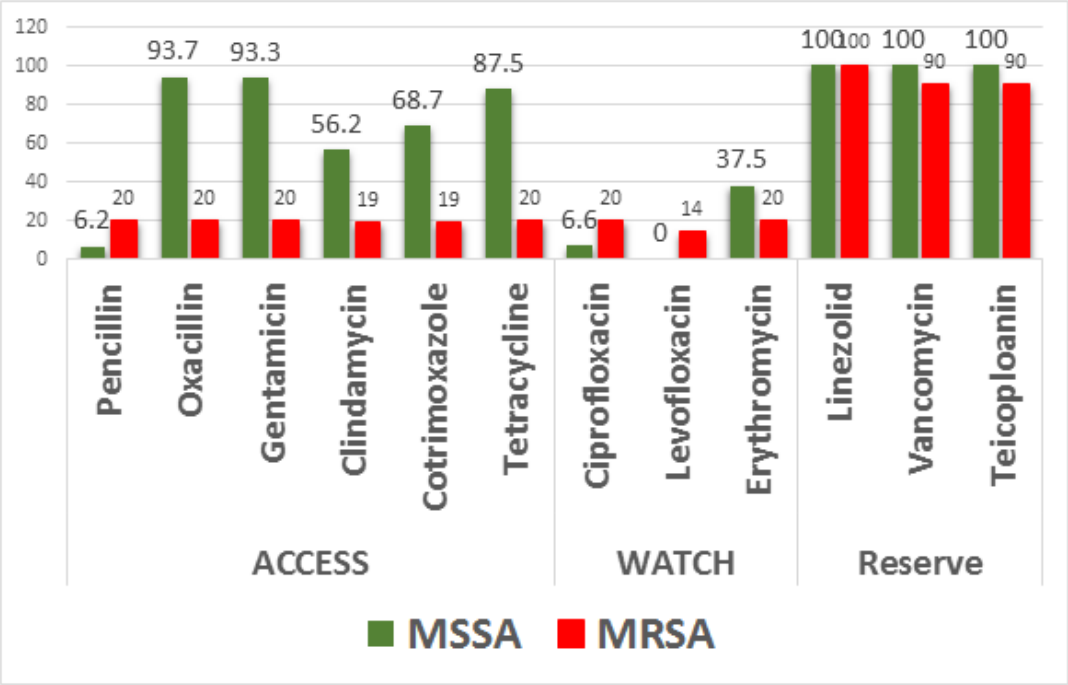

Chart 2: Susceptibility Profile MSSA vs MRSA

A comparison of MSSA and MRSA susceptibility patterns is presented in Chart 2. MSSA isolates demonstrated good susceptibility to Oxacillin, Gentamicin, Cotrimoxazole, Clindamycin, and Tetracycline, reflecting the availability of multiple effective therapeutic options. In contrast, MRSA isolates exhibited resistance to most Access and Watch group antibiotics, with Linezolid (100%), Vancomycin (90%), and Teicoplanin (90%) remaining the only reliably active agents.

DISCUSSION

Regular assessment of antibiogram trends is crucial for identifying the antibiotic-resistance burden faced by a hospital in day-to-day clinical practice. A clinical microbiology laboratory must maintain a structured system for documenting results and periodically sharing them with clinicians to support optimal care. The role of a clinical microbiologist extends beyond providing accurate culture and susceptibility reports; these reports should also act as practical reference tools, enabling clinicians to interpret frequently encountered resistance markers such as MRSA, VRE, CRE, and CRAB. With time, the laboratory should formulate a hospital-specific empirical antibiotic policy that promotes judicious antimicrobial use across departments. Given the dynamic nature of resistance mechanisms, routine compilation and presentation of antibiogram data during infection-control meetings or similar forums is essential, as these patterns often reflect region-specific trends and contribute to global efforts in shaping empiric therapy guidelines. [15, 16] The present study was undertaken to address the lack of local antibiogram information from the Central India region of Madhya Pradesh, a densely populated area with a high infectious-disease burden.

In our analysis, Gram-negative organisms were found to be the predominant bacteria, accounting for almost 76% of all culture-positive samples. This finding is in conformity with that reported by Abebe et al. [17] The distribution of sample types and isolated organisms in our study aligns well with observations reported in several other studies.[18, 19] The isolation of Gram-negative isolates in predominance can be justified in many ways. First, there is the wide spread prevalence of these organisms in the hospital settings. Second is their non-fastidious nature and simple nutritional requirements for growth.

In blood cultures, the most frequently isolated organism was coagulase-negative *Staphylococcus* (CONS). However, the pathogenic significance of CONS is often uncertain unless it is recovered from two separate sites (either two peripheral samples or one central and one peripheral sample). CONS was followed in frequency by *Klebsiella pneumoniae* and *Salmonella Typhi*. *Klebsiella pneumoniae* was predominantly isolated from patients admitted to the IPD and ICU, whereas *Salmonella Typhi* was chiefly detected in samples from OPD patients. Gram-negative sepsis accounted for 66% of all positive blood cultures. In addition, a substantial number of *Candida* species were isolated from blood, all of which carried important clinical relevance. Early initiation of appropriate antifungal therapy—such as fluconazole—plays a critical role in improving patient survival in these cases.

Among the urine samples analyzed, *Escherichia coli* was the most frequently isolated pathogen, accounting for 48% of cases. Previous studies have consistently reported a higher prevalence of *E. coli*, typically ranging from 60% to 85% of all urinary isolates. [20-22] *Enterococcus* spp. emerged as the second most common causative organism of urinary tract infections. Additionally, a notable proportion of ICU patients showed growth of *Klebsiella* spp. and *Pseudomonas aeruginosa* in their urine cultures.

For exudate cultures—including pus, wound swabs, tissue, and biopsy samples—Gram-negative bacteria such as *E. coli* and *Klebsiella pneumoniae* were the most frequently isolated pathogens, followed by *Enterococcus* spp. and *Staphylococcus aureus*. In contrast, several previously published studies have reported *S. aureus* as the predominant pathogen in similar sample types.[23-24] Culture of respiratory specimens demonstrated a markedly high prevalence of Gram-negative bacteria (90%). *Klebsiella pneumoniae* (37%) was the most frequently isolated pathogen, followed by *Acinetobacter* spp. (21%) and *Pseudomonas aeruginosa* (14%). Most respiratory samples—primarily endotracheal aspirates and bronchoalveolar lavage (BAL)—were obtained from ventilated patients, which explains the predominance of these organisms. Singh et al. [25] similarly reported Gram-negative predominance, with *Acinetobacter baumannii* complex (55.5%), *Klebsiella pneumoniae* (38.8%), and *Pseudomonas* spp. (5.5%) As the major pathogens, findings consistent with other published studies from India.[26, 27]

In this study, AST results were reported by listing the pathogenic microorganism followed by the antibiotic susceptibility pattern categorized into **Access, Watch, and Reserve (AWaRe)** groups as recommended by the WHO. This format provides clinicians with a structured, stewardship-oriented interpretation that facilitates the selection of the most appropriate first-line therapy while minimizing unnecessary use of broader-spectrum agents. The **Access** group includes antibiotics that should be preferred as first-line options due to their effectiveness and lower resistance potential, whereas the **Watch** group consists of agents with higher resistance risks that require judicious use. The **Reserve** group comprises last-line antibiotics intended for confirmed or suspected multidrug-resistant infections only. Incorporating AWaRe-based reporting in AST outputs promotes rational antibiotic use, enhances antimicrobial stewardship, and improves clinical decision-making in routine practice.

A high level of antimicrobial resistance was observed among Gram-negative bacterial isolates across nearly all antibiotic classes, as shown in Table 2. *Escherichia coli* remained the predominant uropathogen, with fosfomycin (98%) and nitrofurantoin (81%) retaining excellent activity. Similar high fosfomycin susceptibility has also been reported by Singh et al.[25] For non-urinary *E. coli* infections, piperacillin–tazobactam (58%), cefoperazone–sulbactam (59%), and amikacin (70%) appear to be reasonable empirical options. *Klebsiella pneumoniae* isolates frequently exhibited multidrug-to-extensively drug-resistant profiles. Conventional cephalosporins, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones, and even carbapenems were unreliable for managing serious *Klebsiella* infections. Several pan-drug-resistant isolates required double-disk synergy testing using ceftazidime–avibactam and aztreonam; isolates demonstrating synergy were successfully managed with this combination.

These findings are in line with reports from other regions of India. Batra et al. observed resistance rates surpassing 90% for ampicillin and amoxicillin-clavulanate, and 60–70% for cotrimoxazole and fluoroquinolones among uropathogens.[28] Among the non-fermenters, *Acinetobacter baumannii* exhibited even higher resistance levels than *P. aeruginosa*. CRAB prevalence was notably high, leaving polymyxin B/colistin or ampicillin–sulbactam as the only dependable therapeutic options.

For Gram-positive organisms, the prevalence of MRSA in clinical samples was 68% in our study. This finding is consistent with reports by Anupurba et al. and Tiwari et al., who documented comparable MRSA prevalence rates of 54.85% and 59.3%, respectively.[29, 30] Although some authors have reported comparatively lower MRSA prevalence, all

have emphasized a rising trend in MRSA isolation over recent years. [31] Linezolid and vancomycin remain the most reliable empirical options until AST results are available, with strict de-escalation recommended once susceptibilities are known. Analysis of MSSA–MRSA susceptibility patterns revealed that MRSA isolates displayed high resistance to nearly all oral antimicrobial agents, limiting treatment options to linezolid, vancomycin, and teicoplanin. Conversely, MSSA infections should not be managed with these reserved agents, as they show good susceptibility to safer oral alternatives such as cephalosporins, cotrimoxazole, and clindamycin.

CONCLUSION

The need to highlight these alarm signals of trends of growing antibiotic resistance is vital to the survival of the human race. In the context of limited new antibiotics in the development pipeline and the prolonged timelines required for drug approval, it is essential that existing antimicrobial agents are used judiciously and guided by local antibiogram data to maximize therapeutic outcomes. There is an urgent need to reduce reliance on cephalosporins and other frequently used β-lactam antibiotics, and to reconsider the role of less commonly utilized agents where appropriate. Empirical therapy without microbiological evidence further accelerates resistance and undermines effective patient care; therefore, adherence to evidence-based antimicrobial stewardship practices must become a foundational principle among healthcare professionals. Timely, rational interventions in this direction will contribute significantly to safeguarding the efficacy of available antibiotics for present and future generations.

LIMITATIONS

This study focused on assessing the local antibiotic resistance burden and may not fully reflect resistance patterns observed across other regions of the country. Clinical correlations, including duration of hospital stay, comorbidities, and potential cross-transmission events, were beyond the scope of this analysis. Additionally, the study did not include molecular or genetic characterization of resistance mechanisms, which could have provided deeper insights into the epidemiology of antimicrobial resistance.

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