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Antibiotic Resistance Patterns among Clinical Isolates from Diverse Specimens in Nashik City, Maharashtra: A Cross-Sectional Study

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Abstract

Antimicrobial resistance (AMR) poses a serious threat to effective treatment of bacterial infections, particularly in low- and middle-income countries. Continuous local surveillance of bacterial pathogens and resistance patterns is essential to guide empirical therapy and antimicrobial stewardship. This study aimed to determine the bacterial spectrum and antibiotic resistance patterns among clinical isolates obtained from diverse clinical specimens in Nashik City, Maharashtra. A cross-sectional, laboratory-based study was conducted from January 2023 to December 2024. Clinical specimens including urine, pus, blood, sputum, stool, swabs, catheter tips, pleural fluid, biopsy material, semen, breast milk, endo wash, and bronchial fluid were processed using standard microbiological methods. Bacterial identification was performed using culture characteristics, Gram staining, and biochemical tests as per ICMR guidelines and *Bergey's Manual*. Antibiotic susceptibility testing was carried out using the Kirby–Bauer disk diffusion method following CLSI guidelines. Total of 221 bacterial isolates were recovered. Gram-negative bacteria predominated. *Staphylococcus spp.* (28.96%) was the most frequent isolate, followed by *Escherichia coli* (19.91%) and *Pseudomonas spp.* (12.67%). High resistance was observed to penicillins and cephalosporins. Carbapenem resistance was notable among *Acinetobacter spp.*, while fluoroquinolone resistance was widespread across multiple pathogens. The study highlights a high burden of antimicrobial resistance among clinical isolates in Nashik City, emphasizing the need for routine surveillance, rational antibiotic use, and strengthened antimicrobial stewardship programs.

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Antimicrobial resistance; Clinical isolates; Antibiotic susceptibility; Nashik City; India

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most critical global public health threats of the 21st century, undermining decades of progress in the prevention and treatment of infectious diseases. The World Health Organization (WHO) has identified AMR as a major challenge that threatens the effective management of common bacterial infections, leading to increased morbidity, mortality, and healthcare costs worldwide. Resistant bacterial infections are associated with prolonged hospital stays, increased need for expensive second-line therapies, and higher risk of treatment failure, placing a substantial burden on healthcare systems, particularly in low- and middle-income countries (Prestinaci *et al.,* 2015; WHO, 2018).

The inappropriate, excessive, and often empirical use of antibiotics in both community and hospital settings is a key driver of AMR. Factors such as over-the-counter

availability of antibiotics, lack of adherence to treatment guidelines, self-medication, and inadequate infection control practices contribute significantly to the emergence and dissemination of resistant pathogens. In addition, misuse of antibiotics in veterinary medicine and agriculture further accelerates the spread of resistance across human, animal, and environmental interfaces, highlighting the complexity of AMR as a One Health issue (Van Boeckel *et al.,* 2014; Prestinaci *et al.,* 2015).

India bears a disproportionate burden of antimicrobial resistance due to its high population density, substantial infectious disease load, and widespread antibiotic consumption. Studies have consistently reported high resistance rates among common bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter spp.* Resistance to first-line antibiotics, including β-lactams and fluoroquinolones, is increasingly reported, limiting therapeutic options and necessitating the use of last-resort drugs such as carbapenems and colistin (Laxminarayan *et al.,* 2016; Bonomo *et al.,* 2018). Alarmingly, resistance to these last-line agents has also been documented, particularly among Gram-negative non-fermenters, raising serious concerns about untreatable infections.

Surveillance of bacterial pathogens and their antimicrobial susceptibility profiles is a cornerstone of AMR containment strategies. Clinical microbiology laboratories play a vital role in generating reliable data on pathogen distribution and resistance trends, which are essential for guiding empirical therapy, informing antibiotic stewardship programs, and supporting public health policy formulation. However, AMR patterns vary widely across regions due to differences in healthcare infrastructure, prescribing practices, socioeconomic conditions, and infection control measures. Consequently, local and regional epidemiological studies are indispensable for understanding the true magnitude and dynamics of AMR (Magiorakos *et al.,* 2012; Prestinaci *et al.,* 2015).

Despite national efforts such as the Indian Council of Medical Research (ICMR) Antimicrobial Resistance Surveillance Network, gaps remain in region-specific data, particularly from rapidly urbanizing cities and semi-urban settings. Nashik City, located in Maharashtra, is a fast-growing urban center with a heterogeneous population comprising urban, peri-urban, and rural communities. The city hosts a mix of government hospitals, private healthcare facilities, nursing homes, and diagnostic laboratories catering to diverse patient populations. Such settings are often characterized by high patient turnover and extensive antibiotic use, making them potential hotspots for the emergence and spread of resistant bacteria.

Limited published data are available on the bacterial spectrum and antimicrobial resistance patterns from Nashik City, despite its increasing healthcare demands and expanding population. Understanding local resistance patterns is crucial, as empirical antibiotic therapy is often initiated before culture and susceptibility results are available. In the absence of region-specific data, clinicians may rely on generalized national or international guidelines, which may not accurately reflect local resistance trends and can inadvertently contribute to inappropriate antibiotic selection (Jorgensen & Turnidge, 2015). Clinical specimens such as urine, pus, blood, sputum, stool, swabs, and catheter tips are common sources for isolating pathogenic bacteria responsible for a wide range of infections, including urinary tract infections, bloodstream infections, respiratory tract infections, wound infections, and device-associated infections. Studies have shown that Gram-negative bacteria increasingly dominate clinical isolates, particularly in hospital settings, with high resistance rates to multiple antibiotic classes (Woerther *et al.,* 2013; Bonomo *et al.,* 2018). Simultaneously, Gram-positive pathogens such as *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), continue to pose significant clinical challenges.

The World Health Organization has classified several bacterial pathogens as priority organisms for research and development of new antibiotics, including carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae. Many of these organisms are frequently isolated from clinical specimens in Indian hospitals, emphasizing the urgent need for local surveillance data to guide infection control and antimicrobial stewardship initiatives (WHO, 2017).

In this context, the present cross-sectional study was undertaken to investigate the bacterial spectrum isolated from diverse clinical specimens and to evaluate their antibiotic resistance patterns in Nashik City, Maharashtra. By employing standard microbiological techniques and internationally accepted susceptibility testing methods, this study aims to generate robust region-specific data on clinically relevant bacterial pathogens. The findings are expected to support evidence-based clinical decision-making, optimize

empirical antibiotic therapy, and contribute to local and national efforts to combat antimicrobial resistance.

Materials and Methods

Study Design and Sample Collection

A cross-sectional, laboratory-based descriptive study was conducted in Nashik City, Maharashtra, India, over a two-year period from January 2023 to December 2024. The study aimed to determine the bacterial spectrum and antibiotic resistance patterns among clinical isolates obtained from patients suspected of bacterial infections. Clinical samples were collected from a range of healthcare facilities, including government hospitals, private hospitals, nursing homes, and diagnostic laboratories, to ensure representation of both community-acquired and healthcare-associated infections.

Clinical specimens were collected following standard aseptic procedures by trained healthcare personnel. The samples included urine, pus, blood, sputum, stool, swabs, catheter tips, pleural fluid, biopsy material, semen, breast milk, endo wash, and bronchial fluid. Each specimen was collected in a sterile, properly labeled container and transported promptly to the microbiology laboratory. Samples were processed within 2–4 hours of collection; when immediate processing was not feasible, specimens were stored at 4 °C for short durations to preserve bacterial viability. All procedures were carried out in accordance with ICMR laboratory guidelines for clinical microbiology investigations (ICMR, 2021).

Isolation and Identification of Bacterial Pathogens

Isolation of bacterial pathogens was performed using standard culture techniques. Each clinical sample was inoculated onto Nutrient Agar (NA) for general growth, MacConkey Agar (MA) for selective isolation and differentiation of Gram-negative enteric bacteria, and Xylose Lysine Deoxycholate (XLD) Agar for selective isolation of enteric pathogens such as *Salmonella* and *Shigella*. Inoculated plates were incubated at 37 °C for 18–24 hours under aerobic conditions.

After incubation, plates were examined for colony growth, and colony morphology was recorded based on size, shape, margin, elevation, pigmentation, surface texture, and opacity. Differential reactions on MacConkey and XLD agar, including lactose fermentation and hydrogen sulfide production, were noted for preliminary identification.

Presumptive isolates were subjected to Gram staining for determination of Gram reaction, cell morphology, and cellular arrangement. Definitive identification was carried out using a battery of biochemical tests, including IMViC tests (Indole, Methyl Red, Voges–Proskauer, Citrate utilization), carbohydrate fermentation tests (glucose, lactose, sucrose, mannitol), and additional assays such as catalase, oxidase, motility, bile esculin hydrolysis, and hydrogen sulfide (H_2S) production. The results were interpreted using standard identification keys as described in Bergey's Manual of Systematic Bacteriology and clinical microbiology manuals (Bergey *et al.,* 2012; Forbes *et al.,* 2018).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was performed for all confirmed isolates using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Bacterial suspensions were prepared from fresh cultures and adjusted to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). The standardized inoculum was evenly swabbed onto the surface of Mueller–Hinton agar plates to obtain a uniform lawn culture. Antibiotic discs representing commonly prescribed antimicrobial classes were placed on the inoculated plates. These included penicillins, cephalosporins, carbapenems (imipenem), aminoglycosides (gentamicin and tobramycin), and fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, and sparfloxacin). Plates were incubated at 37 °C for 18–24 hours, after which the diameters of zones of inhibition were measured in millimeters. The results were interpreted as Sensitive (S), Intermediate (I), or Resistant (R) based on CLSI interpretive criteria. Quality control procedures were followed using standard reference strains to ensure accuracy and reproducibility of results (CLSI, 2023; Jorgensen & Turnidge, 2015).

Data Analysis

All microbiological and antibiotic susceptibility data were compiled systematically. Results were expressed as frequencies and percentages. Organism-wise distribution of bacterial isolates and antibiotic-wise resistance patterns were analyzed descriptively to identify predominant pathogens and trends in antimicrobial resistance. The analyzed data were presented in tabular and graphical formats to facilitate interpretation and comparison.

Results and Discussion

A total of 221 bacterial isolates were recovered from various clinical specimens collected during the study period.

Both Gram-positive and Gram-negative bacteria were identified; however, Gram-negative organisms predominated, accounting for approximately 65% of the total isolates, while Gram-positive bacteria constituted about 35%.

Distribution of Bacterial Isolates

Among all isolates, Staphylococcus spp. was the most frequently isolated organism (64 isolates; 28.96%), followed by Escherichia coli (44 isolates; 19.91%) and Pseudomonas spp. (28 isolates; 12.67%). Other commonly isolated organisms included Streptococcus spp. (21 isolates; 9.50%) and Klebsiella spp. (17 isolates; 7.69%). Less frequently isolated pathogens were *Acinetobacter* spp., *Shigella* spp., *Salmonella* spp., *Citrobacter* spp., Proteus spp., *Micrococcus* spp., and *Enterococcus* spp.

Table.1 Organism-wise Distribution of Bacterial Isolates (n = 221)

Bacterial Isolate	Number of Isolates	Percentage (%)
Staphylococcus spp.	64	28.96
Escherichia coli	44	19.91
Pseudomonas spp.	28	12.67
Streptococcus spp.	21	9.50
Klebsiella spp.	17	7.69
Acinetobacter spp.	14	6.33
Shigella spp.	11	4.98
Salmonella spp.	9	4.07
Citrobacter spp.	2	0.90
Proteus spp.	4	1.81
Micrococcus spp.	4	1.81
Enterococcus spp.	5	2.26
Total	221	100.00

Sample-wise Distribution of Isolates

Analysis of specimen types revealed that urine samples yielded the highest number of isolates (46; 20.81%), followed by pus (38; 17.19%), endo wash (30; 13.57%), and stool samples (29; 13.12%). Sputum (23; 10.41%) and swab samples (19; 8.60%) also contributed substantially. Blood samples yielded comparatively fewer isolates (10; 4.52%). The least number of isolates were obtained from pleural fluid, biopsy material, breast milk, bronchial fluid, and catheter tips.

Table.2 Sample-wise Distribution of Bacterial Isolates

Clinical Sample	Number of Isolates	Percentage (%)
Urine	46	20.81
Pus	38	17.19
Endo wash	30	13.57
Stool	29	13.12
Sputum	23	10.41
Swab	19	8.60
Blood	10	4.52
Semen	8	3.62
Bronchial fluid	7	3.17
Pleural fluid	3	1.36
Biopsy material	2	0.90
Breast milk	2	0.90
Catheter tip	2	0.90
Total	221	100.00

Antibiotic Resistance Patterns

Antibiotic susceptibility testing demonstrated high levels of resistance across multiple antibiotic classes. Resistance to penicillins and cephalosporins was widespread among both Gram-positive and Gram-negative organisms. *Escherichia coli* showed very high resistance to cephalosporins (92%), indicating a strong likelihood of extended-spectrum β-lactamase (ESBL) production. Resistance to fluoroquinolones was also notable in *E. coli*, particularly against ciprofloxacin and ofloxacin. *Acinetobacter* spp. exhibited marked resistance to imipenem (64%), along with high resistance to ciprofloxacin (78%) and gentamicin (67%), highlighting its multidrug-resistant nature. Pseudomonas spp. demonstrated moderate to high resistance to fluoroquinolones and aminoglycosides, whereas Staphylococcus spp. showed considerable resistance to penicillin and fluoroquinolones but comparatively lower resistance to carbapenems.

Table.3 Percentage Resistance of Major Bacterial Isolates to Selected Antibiotics

Organism	Penicillin	Cephalosporin	Imipenem	Gentamicin	Ciprofloxacin
Staphylococcus spp.	72	61	30	48	35
Escherichia coli	52	92	45	48	33
Acinetobacter spp.	54	57	64	67	78
Pseudomonas spp.	37	28	45	56	63
Klebsiella spp.	19	28	21	23	13

Tthe results indicate a predominance of Gram-negative pathogens, particularly enteric bacteria and non-fermenters, with high resistance to commonly prescribed antibiotics. Urinary tract infections were the most common source of isolates, and multidrug resistance was especially evident among *Acinetobacter spp.*, *E. coli*, and *Pseudomonas spp.*

The present cross-sectional study provides comprehensive insight into the bacterial spectrum and antibiotic resistance patterns among clinical isolates obtained from diverse specimens in Nashik City, Maharashtra. The findings reveal a predominance of Gram-negative bacteria, a trend increasingly reported from both community and hospital settings in India and other low- and middle-income countries. This shift toward Gram-negative pathogens poses significant therapeutic challenges due to their intrinsic and acquired resistance mechanisms.

In the current study, Gram-negative bacteria constituted approximately two-thirds of all isolates, with *Escherichia coli* and *Pseudomonas spp.* emerging as major pathogens. Similar predominance of Gram-negative organisms has been reported in studies from different regions of India, reflecting changing epidemiological trends in bacterial infections (Laxminarayan *et al.,* 2016; Woerther *et al.,* 2013). The high frequency of *E. coli*, particularly from urine samples, underscores its established role as the leading cause of urinary tract infections and highlights the burden of uropathogenic strains in clinical practice.

Among Gram-positive bacteria, *Staphylococcus spp.* was the most frequently isolated organism. This finding is consistent with earlier reports that identify staphylococci as major contributors to skin and soft tissue infections, wound infections, and device-associated infections. The persistence of *Staphylococcus spp.* as a dominant pathogen emphasizes its adaptability and ability to survive in diverse clinical environments (Tong *et al.,* 2015).

Sample-wise analysis showed that urine samples yielded the highest number of isolates, followed by pus and endo wash samples. This distribution reflects the high incidence of urinary tract infections and wound-related infections in routine clinical settings. Similar sample-wise patterns have been documented in multiple hospital-based surveillance studies, reinforcing the clinical relevance of these specimen types in AMR monitoring (Flores-Mireles *et al.,* 2015).

Antibiotic susceptibility testing revealed widespread resistance to penicillins and cephalosporins across most bacterial species. Notably, *E. coli* exhibited extremely high resistance to cephalosporins, strongly suggesting the presence of extended-spectrum β-lactamase (ESBL)-producing strains. ESBL-mediated resistance among Enterobacteriaceae has become a major concern in India, significantly limiting the effectiveness of commonly prescribed β-lactam antibiotics and leading to increased reliance on carbapenems (Woerther *et al.,* 2013; Bonomo *et al.,* 2018). Carbapenem resistance observed in

Acinetobacter spp. is particularly alarming. *Acinetobacter baumannii* is recognized globally as a critical priority pathogen by the World Health Organization due to its capacity to acquire resistance to nearly all available antibiotics. The high resistance to imipenem and fluoroquinolones observed in this study mirrors findings from other Indian and international studies and highlights the growing threat posed by multidrug-resistant non-fermenters in healthcare settings (Howard *et al.,* 2012; Bonomo *et al.,* 2018).

Fluoroquinolone resistance was widespread across both Gram-positive and Gram-negative organisms, including *E. coli*, *Pseudomonas spp.*, and *Staphylococcus spp.* This pattern likely reflects the extensive empirical and sometimes inappropriate use of fluoroquinolones in outpatient and inpatient care. Previous studies have consistently linked high fluoroquinolone consumption with increased resistance rates, underscoring the need for more judicious use of this antibiotic class (Prestinaci *et al.,* 2015).

The resistance trends observed in this study have important clinical implications. High resistance to first-line antibiotics reduces treatment options, increases the risk of therapeutic failure, and contributes to prolonged hospital stays and higher healthcare costs. These findings emphasize the importance of culture-based diagnosis and susceptibility-guided therapy, particularly in regions with high AMR burden.

While the study provides valuable region-specific data, it has certain limitations. Molecular characterization of resistance mechanisms, such as confirmation of ESBL or carbapenemase production, was not performed due to resource constraints. Additionally, clinical outcome data were not included, which could have further strengthened the correlation between resistance patterns and patient prognosis.

Despite these limitations, the study contributes meaningful data to the existing body of AMR surveillance in India. The findings reinforce the urgent need for continuous local surveillance**,** strict adherence to antibiotic stewardship principles, and strengthening of infection control practices. Generating and utilizing such region-specific data is essential for optimizing empirical therapy and for informing public health strategies aimed at curbing the spread of antimicrobial resistance.

This study demonstrates a diverse bacterial spectrum among clinical isolates in Nashik City, with a predominance of Gram-negative pathogens and high levels of antimicrobial resistance. Widespread resistance to penicillins, cephalosporins, and fluoroquinolones was observed, while notable carbapenem resistance among *Acinetobacter spp.* highlights a serious therapeutic challenge. These findings emphasize the importance of culture-guided therapy, routine antibiotic susceptibility testing, and continuous local surveillance**.** Implementation of antimicrobial stewardship programs and strict infection control measures is urgently required to promote rational antibiotic use, limit the spread of resistant pathogens, and support effective clinical management and public health interventions.

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