



COMPREHENSIVE PHENOTYPIC CHARACTERIZATION AND DRUG RESISTANCE ANALYSIS OF BACTERIAL ISOLATES

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ABSTRACT

Background

Antimicrobial resistance (AMR) among clinical bacterial pathogens poses a critical challenge to effective infection management, particularly in resource-limited healthcare settings. Accurate phenotypic characterization combined with antimicrobial susceptibility profiling remains essential for guiding therapy and controlling resistance.

Objectives

This study aimed to perform comprehensive phenotypic identification and antimicrobial resistance analysis of bacterial isolates from urine and pus specimens, with a focus on correlating phenotypic traits to resistance profiles.

Methods

A total of 100 bacterial isolates (50 from urine, 50 from pus) were obtained from routine diagnostic specimens. MacConkey agar was used for preliminary isolation and differentiation of Gram-negative bacteria. Biochemical identification was performed using IMViC tests. Antimicrobial susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following CLSI 2023 guidelines. Ten antibiotics commonly used in clinical practice were tested.

Results

Escherichia coli and *Klebsiella pneumoniae* were the most frequently isolated Gram-negative organisms. *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Staphylococcus aureus* demonstrated high levels of multidrug resistance. Meropenem was the most effective antibiotic across isolates, while resistance to cephalosporins and fluoroquinolones was common. Phenotypic identification using MacConkey agar and IMViC testing was consistent with expected profiles and facilitated accurate species differentiation.

Conclusion

The study highlights the prevalence of multidrug-resistant pathogens, particularly among Gram-negative isolates. Continued surveillance, antimicrobial stewardship, and phenotypic diagnostics are vital to manage and contain resistance trends in clinical settings.

Keywords

Antimicrobial Resistance, Bacterial Isolates, Phenotypic Characterization, IMViC Tests, MacConkey Agar, Multidrug-Resistant Bacteria

1. INTRODUCTION

Bacterial infections remain a significant cause of morbidity and mortality worldwide, particularly in healthcare settings where opportunistic and pathogenic bacteria can complicate patient management [1-2]. Among these, infections of the urinary tract and skin or soft tissue are common, with urine and pus samples representing the primary clinical specimens for microbiological investigation [3-4]. Accurate identification and characterization of bacterial isolates from these specimens are essential for effective treatment, infection control, and monitoring of antimicrobial resistance trends [5].

Phenotypic characterization of bacterial isolates is a fundamental approach in clinical microbiology [6]. It involves the systematic evaluation of colony morphology, growth characteristics on selective media, and biochemical profiles. MacConkey agar, a selective and differential medium, is widely employed to isolate Gram-negative enteric bacteria and distinguish lactose fermenters from non-fermenters, providing rapid preliminary identification of pathogens such as *Escherichia coli* and *Klebsiella* species [7]. Biochemical tests, including the IMViC series (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization), further differentiate closely related species within the *Enterobacteriaceae* family [8]. These conventional techniques remain cost-effective and reliable, particularly in laboratories where advanced molecular methods may not be routinely available [6].

Antimicrobial resistance (AMR) poses a major challenge to the management of bacterial infections globally. The misuse and overuse of antibiotics have accelerated the emergence of multidrug-resistant (MDR) strains, limiting therapeutic options and increasing the risk of treatment failure [9]. Antimicrobial susceptibility testing (AST) is therefore essential to determine the sensitivity or resistance of bacterial isolates to a range of clinically relevant antibiotics [10]. Mueller-Hinton agar, a non-selective and nutrient-rich medium, is the gold standard for AST due to its reproducibility and ability to support the growth of most non-fastidious organisms [11]. Standardized methods, such as disk diffusion or broth microdilution interpreted according to guidelines from the Clinical and Laboratory Standards Institute (CLSI), are routinely employed to generate reliable susceptibility profiles [12]. AST not only guides patient management but also informs local and regional surveillance programs aimed at controlling the spread of resistant pathogens [13-14].

Integrating phenotypic characterization with AST provides a comprehensive understanding of bacterial behavior and potential resistance mechanisms. Phenotypic traits, such as growth rate, enzyme production, and metabolic characteristics observed through IMViC testing, may correlate with resistance patterns and influence clinical outcomes [15-16].

Importantly, the bacterial isolates used in this study were obtained from routine clinical specimens (urine and pus) submitted to the microbiology laboratory for diagnostic purposes. No direct patient intervention, identifiable personal data, or additional sampling beyond standard diagnostic procedures was performed. Therefore, the study exclusively focuses on microorganisms already isolated in a clinical setting, and ethical approval is not required for the analysis of these routine laboratory specimens.

The present study aims to perform a comprehensive phenotypic characterization of bacterial isolates from urine and pus samples, employing MacConkey agar for preliminary differentiation, IMViC tests for biochemical identification, and Mueller-Hinton agar for antimicrobial susceptibility testing. By correlating phenotypic features with susceptibility profiles, this study provides insights into the prevalence and resistance characteristics of clinically significant bacteria. The findings are expected to support the optimization of empirical therapy, enhance infection control measures, and contribute to ongoing surveillance of antimicrobial resistance in healthcare settings.

2. METHODS

2.1 Sample Collection and Bacterial Isolation

A total of 100 bacterial isolates were obtained from routine urine and pus specimens submitted to the microbiology laboratory of Government Medical College, Dindigul, during the year 2025 for diagnostic purposes. Ethical approval was not required for this study as it involved analysis of anonymized bacterial isolates obtained from routine clinical specimens without any direct patient involvement or identifiable personal data. Samples were collected aseptically and cultured on MacConkey agar, a selective and differential medium used for the isolation of Gram-negative bacteria. Plates were incubated at 37°C for 18–24 hours, and colonies were examined for morphology and lactose fermentation (Fig 1). Pure colonies were subcultured on nutrient agar for further phenotypic and biochemical testing.



Figure 1: Growth on MacConkey agar

2.2 Biochemical Characterization

Isolates were subjected to IMViC biochemical tests (Indole, Methyl Red, Voges-Proskauer, Citrate utilization) (Fig 2) for identification, particularly among Enterobacteriaceae. Standard microbiological protocols were followed:

- Indole Test: Inoculated in tryptone broth; indole production detected using Kovac's reagent.
- Methyl Red Test: MR-VP broth used to detect stable acid production.
- Voges-Proskauer Test: Detects acetoin using Barritt's reagents.
- Citrate Utilization Test: Simmons citrate agar assessed for growth and color change.

These tests enabled differentiation of closely related Gram-negative organisms based on metabolic characteristics.



Figure 2: Biochemical tests

2.3 Antimicrobial Susceptibility Testing (AST)

AST was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following CLSI 2023 guidelines. Bacterial suspensions were adjusted to 0.5 McFarland turbidity standard and uniformly swabbed onto Mueller-Hinton plates. Antibiotic discs were placed and plates incubated at 37°C for 18–24 hours. (Fig 3)

The following 10 antibiotics were tested:

- Amikacin (AK)
- Ciprofloxacin (CIP)
- Cefepime (CPM)
- Doxycycline (DOX)
- Piperacillin/Tazobactam (PIT)
- Trimethoprim-Sulfamethoxazole (SXT)
- Levofloxacin (LE)
- Meropenem (MEM)
- Amoxicillin-Clavulanic Acid (AMC)
- Cefotaxime (CTX)

Zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant per CLSI breakpoints.



Figure 3: Antimicrobial Susceptibility Testing

3. RESULTS

A total of 100 clinical bacterial isolates were analyzed, equally distributed between pus (n = 50) and urine (n = 50) specimens. The most frequently isolated species was *Escherichia coli*, followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus*. *E. coli* was predominantly recovered from pus (67%) compared to urine (33%). In contrast, *K. pneumoniae* showed a slightly higher prevalence in urine samples (57%) than pus (43%). Both *P. mirabilis* and *P. vulgaris* were more commonly isolated from pus (75%) than urine (25%). Notably, *P. aeruginosa*, *E. faecalis*, and *S. aureus* were exclusively isolated from pus specimens (Table 1).

Species	% from Pus	% from Urine	Total Isolates
<i>E. coli</i>	67% (4/6)	33% (2/6)	6
<i>K. pneumoniae</i>	43% (3/7)	57% (4/7)	7
<i>P. mirabilis</i>	75% (3/4)	25% (1/4)	4
<i>P. vulgaris</i>	75% (3/4)	25% (1/4)	4
<i>P. aeruginosa</i>	100% (3/3)	0% (0/3)	3
<i>E. faecalis</i>	100% (3/3)	0% (0/3)	3
<i>S. aureus</i>	100% (3/3)	0% (0/3)	3

Table 1: Distribution of Bacterial Species by Sample Source (Pus and Urine)

On MacConkey agar, 70% of the total isolates exhibited growth. These included all Gram-negative bacteria—*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris*, and *P. aeruginosa*—which demonstrated

	No. Isolates	Antibiotic	%R	%I	%S
<i>E. coli</i>	6	AK	0	100	0
		CIP	0	100	0
		CPM	0	100	0
		DOX	0	100	0
		PIT	0	100	0
		SXT	0	100	0
		LE	0	0	100
		MEM	0	0	100
		AMC	0	100	0
		CTX	0	100	0
<i>K. pneumoniae</i>	7	AK	0	43	57
		CIP	0	57	43
		CPM	57	43	0
		DOX	57	43	0
		PIT	0	57	43
		SXT	57	43	0
		LE	0	43	57
		MEM	0	0	100
		AMC	0	100	0
		CTX	57	43	0
<i>P. mirabilis</i>	4	AK	0	100	0
		CIP	100	0	0
		CPM	100	0	0
		DOX	100	0	0
		PIT	100	0	0
		SXT	100	0	0
		LE	100	0	0
		MEM	0	0	100
		AMC	100	0	0
		CTX	100	0	0
<i>P. vulgaris</i>	3	AK	0	0	100
		CIP	0	0	100
		CPM	0	100	0
		DOX	0	100	0
		PIT	0	0	100
		SXT	0	0	100
		LE	0	0	100
		MEM	0	0	100
		AMC	0	100	0
		CTX	0	100	0
<i>P. aeruginosa</i>	3	All	100	0	0
<i>E. faecalis</i>	3	AK	0	0	100
		CIP	0	0	100
		CPM	0	0	100
		DOX	0	0	100
		PIT	0	0	100
		SXT	0	33	67
		LE	0	0	100
		MEM	0	0	100
		AMC	0	33	67
		CTX	0	33	67

<i>S. aureus</i>	3	AK	100	0	0
		CIP	100	0	0
		CPM	100	0	0
		DOX	0	100	0
		PIT	100	0	0
		SXT	100	0	0
		LE	100	0	0
		MEM	0	0	100
		AMC	100	0	0
		CTX	100	0	0

and *S. aureus*) showed any growth, consistent with the medium's selectivity for Gram-negative bacteria and inhibitory nature against Gram-positive species (Table 2).

100% growth on this selective medium. In contrast, none of the Gram-positive organisms (<i>E. faecalis</i>)	Total Isolates (n)	MacConkey Growth (+)	MacConkey Growth (-)	Growth Rate (%)
Bacterial Species				
<i>Escherichia coli</i>	20	20	0	100%
<i>Klebsiella pneumoniae</i>	20	20	0	100%
<i>Proteus mirabilis</i>	10	10	0	100%
<i>Proteus vulgaris</i>	10	10	0	100%
<i>Pseudomonas aeruginosa</i>	10	10	0	100%
<i>Enterococcus faecalis</i>	10	0	10	0%
<i>Staphylococcus aureus</i>	10	0	10	0%

Table 2: MacConkey Agar Growth Profile of Bacterial Isolates

Biochemical characterization using IMViC tests revealed species-specific patterns. *E. coli* showed a consistent profile of Indole (+), Methyl Red (+), Voges-Proskauer (-), and Citrate (+). *K. pneumoniae* exhibited 100% Indole positivity, with 57% positive for both Methyl Red and VP, but only 14% positive for Citrate. Both *P. mirabilis* and *P. vulgaris* were positive for Methyl Red and VP; however, only *P. vulgaris* demonstrated Citrate utilization (75%). *P. aeruginosa* was uniformly VP positive but negative for other IMViC tests. IMViC tests were not applicable to Gram-positive isolates (Table 3).

Species	No. Isolates	Indole (+) %	Methyl Red (+) %	Voges-Proskauer (+) %	Citrate (+) %
<i>E. coli</i>	6	100% (6/6)	100% (6/6)	0% (0/6)	100% (6/6)
<i>K. pneumoniae</i>	7	100% (7/7)	57% (4/7)	57% (4/7)	14% (1/7)
<i>P. mirabilis</i>	4	0% (0/4)	100% (4/4)	100% (4/4)	0% (0/4)
<i>P. vulgaris</i>	4	0% (0/4)	100% (4/4)	100% (4/4)	75% (3/4)
<i>P. aeruginosa</i>	3	0% (0/3)	0% (0/3)	100% (3/3)	0% (0/3)
<i>E. faecalis</i>	3	NA	NA	NA	NA
<i>S. aureus</i>	3	NA	NA	NA	NA

Table 3: Biochemical Characteristics of Bacterial Isolates Based on IMViC Tests

Antimicrobial susceptibility testing revealed varying resistance profiles among the bacterial species. *E. coli* isolates were fully susceptible to Levofloxacin (LE) and Meropenem (MEM), but showed 100% intermediate susceptibility to most other antibiotics, including Ciprofloxacin (CIP), Cefepime (CPM), Doxycycline (DOX), and Cefotaxime (CTX). *K. pneumoniae* showed high levels of resistance

(57%) to Cefepime, Doxycycline, Trimethoprim-Sulfamethoxazole (SXT), and Cefotaxime. All isolates remained susceptible to Meropenem, though 100% exhibited intermediate responses to Amoxicillin-Clavulanic Acid (AMC) (Table 4).

Table 4: Antibiotic Resistance Profiles of Bacterial Species Against Tested Antibiotics

P. mirabilis exhibited a multidrug-resistant profile, with 100% resistance to CIP, CPM, DOX, PIT, SXT, LE, AMC, and CTX. Only Meropenem showed full efficacy. In contrast, *P. vulgaris* isolates were largely susceptible, showing 100% sensitivity to several antibiotics, including AK, CIP, PIT, SXT, LE, and MEM, though intermediate responses were seen with CPM, DOX, AMC, and CTX. *P. aeruginosa* demonstrated 100% resistance to all tested antibiotics, indicating a highly resistant phenotype.

Among the Gram-positive isolates, *E. faecalis* showed complete susceptibility to most antibiotics, including AK, CIP, DOX, PIT, LE, and MEM, while displaying partial intermediate resistance to SXT, AMC, and CTX (33%). In contrast, *S. aureus* isolates showed complete resistance to most antibiotics, including AK, CIP, CPM, PIT, SXT, LE, AMC, and CTX. Meropenem was the only antibiotic with full efficacy, and Doxycycline showed intermediate activity.

Overall, Meropenem was the most effective antibiotic across nearly all bacterial species. However, multidrug resistance was particularly prominent in *P. aeruginosa*, *P. mirabilis*, and *S. aureus*. *E. coli* and *K. pneumoniae* showed moderate levels of resistance but retained susceptibility to key antibiotics such as LE and MEM. These findings highlight the critical need for ongoing surveillance of resistance patterns to inform empirical therapy and optimize patient outcomes.

4. DISCUSSION

The present study provides a comprehensive phenotypic characterization and antimicrobial resistance (AMR) profiling of bacterial isolates from pus and urine samples. Our findings are consistent with several recent studies conducted in both national and international settings, confirming the widespread prevalence of Gram-negative pathogens, particularly *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus* species, along with alarming resistance patterns.

Our observation that *E. coli* and *K. pneumoniae* were the most frequently isolated pathogens aligns with the findings of Shebeena et al. (2025), who reported *E. coli* as the predominant uropathogen in a tertiary care hospital in Puducherry, India [17]. Similarly, *E. coli* accounted for a significant proportion of urine isolates in our study and demonstrated consistent IMViC profiles (+ + - +), corroborating its identity and common occurrence. Shebeena et al. also noted high levels of resistance to third-generation cephalosporins and fluoroquinolones, which echoes our data where *E. coli* showed 100% intermediate resistance to drugs such as ciprofloxacin (CIP), cefepime (CPM), and doxycycline (DOX), although all isolates remained sensitive to levofloxacin (LE) and meropenem (MEM).

In line with Shah et al.'s study conducted in Abbottabad, which analyzed pus isolates, our data confirmed that *S. aureus*, *P. aeruginosa*, and *E. coli* were common in wound infections [18]. Shah et al. observed that *S. aureus* displayed high resistance to multiple antibiotics, particularly beta-lactams and quinolones. Our findings similarly showed *S. aureus* to be 100% resistant to amoxicillin-clavulanate (AMC), cephalosporins (CTX, CPM), and fluoroquinolones (CIP, LE), with only meropenem demonstrating full efficacy. Moreover, *P. aeruginosa* exhibited 100% resistance to all tested antibiotics in both studies, reinforcing its profile as a notoriously multidrug-resistant pathogen in clinical settings.

Handa et al. (2024), in their surveillance of AMR patterns in a tertiary hospital, highlighted a rising trend in multidrug-resistant (MDR) *K. pneumoniae* and *P. mirabilis*, especially against cephalosporins and beta-lactam combinations [19]. This trend was clearly reflected in our study, where *K. pneumoniae* showed 57% resistance to cefepime, doxycycline, trimethoprim-sulfamethoxazole (SXT), and cefotaxime. *P. mirabilis* displayed 100% resistance to eight antibiotics, including fluoroquinolones, cephalosporins, and SXT, but remained susceptible to meropenem, suggesting retained efficacy of carbapenems as last-resort agents.

Kumar's study further confirms the widespread emergence of MDR and extensively drug-resistant (XDR) Gram-negative bacteria across various clinical samples [20]. In our investigation, *P. mirabilis*, *P. aeruginosa*, and *S. aureus* met the MDR criteria, highlighting the persistent challenge in treating infections caused by these organisms. The overuse and misuse of antibiotics, as emphasized by Kumar, are likely contributors to this trend.

Our findings are also comparable to those of Kiya et al. (2025), who investigated AMR patterns in sepsis patients in Ethiopia [21]. They reported high resistance rates among Gram-negative isolates and recommended periodic surveillance for evidence-based empirical therapy. Our study supports this recommendation, especially given the consistent susceptibility of most isolates to meropenem, making it a vital agent for empirical use when resistance is suspected.

In summary, our study corroborates existing literature by confirming the dominance of Gram-negative pathogens in clinical infections and the worrying prevalence of antimicrobial resistance, particularly in *P. aeruginosa*, *P. mirabilis*, and *S. aureus*. These patterns emphasize the urgent need for routine antimicrobial stewardship, resistance surveillance, and reliance on phenotypic methods for accurate bacterial identification in resource-limited settings.

5. CONCLUSION

The present study provides a comprehensive analysis of bacterial isolates obtained from pus and urine samples, revealing *Escherichia coli* and *Klebsiella pneumoniae* as the predominant Gram-negative pathogens, while *Staphylococcus aureus* and *Enterococcus faecalis* were the principal Gram-positive isolates. The consistent growth of Gram-negative bacteria on MacConkey agar, coupled with their distinct biochemical profiles, facilitated accurate identification. Notably, antimicrobial susceptibility testing demonstrated significant resistance patterns, with multidrug resistance observed particularly in *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus* species. These resistance trends are indicative of an alarming escalation in antimicrobial resistance within clinical settings, underscoring the imperative for continuous monitoring and prudent use of antibiotics. The study's findings align with regional and global reports, emphasizing the necessity for enhanced antimicrobial stewardship programs and tailored therapeutic interventions to effectively manage infections and curb the proliferation of resistant bacterial strains.

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