RESEARCH PAPER

Organism- and Specimen-Specific Antimicrobial Resistance Patterns and Multidrug-Resistance Profiles of Clinical Gram-Negative Bacterial Isolates in Meerut, India

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ABSTRACT
Antimicrobial resistance in Gram-negative bacteria is a major clinical problem. *Escherichia coli* is one of the most important organisms in this group because it causes urinary tract infection, bloodstream infection, wound infection and other extraintestinal infections. The present work evaluated the phenotypic identification and antimicrobial susceptibility profile of clinical Gram-negative bacterial isolates, with focus on *E. coli*. A total of 440 bacterial isolates were collected from a diagnostic laboratory in Meerut between 2022 and 2025. Of these, 360 Gram-negative bacterial isolates were included in the study. Identification was performed by culture, microscopy, biochemical testing and the VITEK 2 Compact system. Antimicrobial susceptibility testing was performed by the Kirby Bauer disk diffusion method using CLSI breakpoints, with automated AST for selected multidrug-resistant isolates. Urine was the largest sample group with 200 isolates (55.6%), followed by pus with 96 isolates (26.7%). *E. coli* was the dominant organism and represented 222 of 360 isolates. Norfloxacin and ceftriaxone showed the highest resistance rates, while lower resistance was observed against colistin, fosfomycin and tigecycline. *E. coli* contributed the largest absolute number of multidrug-resistant isolates, while Acinetobacter spp., Proteus spp. and Klebsiella spp. showed higher proportional MDR burdens. The findings show a substantial phenotypic resistance burden against fluoroquinolones and third-generation cephalosporins and support routine organism-level antimicrobial resistance surveillance in the local clinical setting.
Keywords: *Escherichia coli*; Gram-negative bacteria; phenotypic identification; antimicrobial susceptibility; multidrug resistance; clinical isolates; Meerut.

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INTRODUCTION

Escherichia coli is a Gram-negative, rod-shaped and facultative anaerobic bacterium. It belongs to the family Enterobacteriaceae. It grows well on routine culture media and is commonly recovered from the intestinal tract of humans and animals. It is also one of the best-studied bacteria in microbiology and molecular biology [1,2].

The importance of *E. coli* comes from its dual nature. Many strains live as normal gut bacteria and do not cause disease. Other strains cause intestinal infection, urinary tract infection, bloodstream infection, neonatal meningitis and other extraintestinal infections. This wide disease range makes *E. coli* a useful organism for studying both commensal life and bacterial pathogenesis [1,3,4].

E. coli is commonly described as a lactose-fermenting member of the Enterobacteriaceae. In the laboratory, it is usually identified by culture, biochemical reactions and automated identification systems. These methods remain important because they confirm the bacterial species and guide the first clinical interpretation [1,5].

E. coli is one of the most frequent bacterial causes of human infection. It is common in urinary tract infection, diarrheal disease and bloodstream infection. Its clinical impact is increased by recurrence, treatment failure and antimicrobial resistance. This makes *E. coli* important in both hospital and community settings [5-7].

Extraintestinal pathogenic *E. coli*, or ExPEC, includes strains that cause infections outside the intestine. Uropathogenic *E. coli* is one of the best studied ExPEC groups. UPEC is the leading bacterial cause of urinary tract infection and is also linked with recurrence, treatment failure and antimicrobial resistance [4-6].

Antimicrobial resistance is now a major part of *E. coli* clinical importance. Resistant strains reduce treatment options and increase the burden of infection. *E. coli* is also included among major organisms contributing to the global burden of bacterial antimicrobial resistance [6,7].

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The objective of this work was to characterize the distribution and antimicrobial susceptibility profile of clinical Gram-negative bacterial isolates, with particular focus on *Escherichia coli*, and to determine the burden and pattern of multidrug resistance across the major clinical sample types.

MATERIALS AND METHODS

Sample Collection

Clinical bacterial isolates were collected from a diagnostic laboratory in Meerut between 2022 and 2025. The specimens were received from regional hospitals and clinics and included urine, blood, sputum, pus, semen, cerebrospinal fluid and other clinically indicated samples. Specimens were collected in sterile containers, swabs or aerobic blood-culture bottles, as appropriate. Midstream clean-catch urine was collected after instructing the patients, and all specimens were labelled and transported under appropriate conditions.

Bacterial Identification

Bacterial isolates were identified by culture characteristics, microscopy, conventional biochemical testing and, for selected isolates, the VITEK® 2 Compact automated identification system (bioMérieux).

Media Preparation

Dehydrated culture media were prepared according to the manufacturer's instructions. The required quantity was suspended in distilled water, heated with intermittent mixing until completely dissolved and sterilized at 121°C and 15 psi for 15 minutes. The sterilized medium was cooled to approximately 45°C and poured aseptically into sterile 90-mm Petri dishes.

After solidification, each batch was incubated for sterility and quality control. Contaminated plates were discarded. Culture media were obtained from HiMedia Laboratories, whereas aerobic blood-culture bottles were obtained from Becton Dickinson.

Culturing and Incubation

Urine specimens were inoculated with a calibrated 10-µL loop by the semi-quantitative streaking method, mainly on cystine-lactose-electrolyte-deficient agar. Blood specimens were inoculated into BD BACTEC aerobic bottles and processed in the BACTEC 9240 system, followed by subculture after growth detection. Sputum, pus and other specimens were streaked on appropriate media, including blood agar and MacConkey agar. Plates were incubated aerobically at 37°C for 24–48 hours. Plates without visible growth at 24 hours were incubated for an additional 24 hours, and pure colonies were selected for microscopy, biochemical identification and antimicrobial susceptibility testing.

Morphological Identification

After incubation, the plates were examined for colony count, size, shape, pigmentation, mucoid appearance and lactose fermentation on MacConkey and CLED agar. Urine colony counts of 10^3–10^5 CFU/mL were interpreted in relation to the specimen type and available clinical information because clinically relevant bacteriuria may occur below the classical threshold of 10^5 CFU/mL [8]. Preliminary identification was based on colony morphology and was confirmed by microscopy and biochemical reactions. Selected isolates were also identified by the VITEK® 2 Compact system.

Microscopy

Gram staining was performed on pure colonies to determine bacterial morphology and Gram reaction [9]. A thin smear was prepared on a grease-free glass slide, air-dried, heat-fixed and stained by the standard Gram-staining procedure. The stained smear was examined under the oil-immersion objective, and the bacterial shape, arrangement and Gram reaction were recorded.

Biochemical Testing

Conventional biochemical tests were performed according to standard identification procedures [10]. The tests included catalase, oxidase, citrate utilization, urease, indole production and motility, as required for each isolate. The expected reactions used for preliminary differentiation are summarized in Table 1.

For the catalase test, a pure colony was exposed to hydrogen peroxide on a clean glass slide. Immediate bubble formation was recorded as a positive reaction [10].

For the oxidase test, a pure colony was rubbed onto reagent-impregnated filter paper. Development of a dark purple colour within the specified reaction time was recorded as positive [10].

Citrate utilization was tested on Simmons citrate agar slants. Visible growth with a colour change from green to blue was interpreted as a positive reaction [10].

Urease production was tested on urea agar slants. Development of a pink or magenta colour, resulting from ammonia production and an increase in pH, was recorded as positive [10].

Indole production was tested after overnight growth in tryptone broth. Kovac's reagent was added, and formation of a red layer at the surface was recorded as positive [10]. Incubation was carried out aerobically at 37°C.

Motility was examined by the hanging-drop method using a cavity slide. Directional movement distinct from Brownian movement and fluid currents was recorded as true motility [10]. Swarming growth on agar was additionally considered for the identification of *Proteus spp.*

Table 1: Summary of Key Morphological and Biochemical Tests Used for Laboratory Identification of Bacterial Isolates

Test	*E. coli*	*Klebsiella*	*Proteus*	*Pseudomonas*	*Acinetobacter*
Gram stain	GNB	GNB	GNB	GNB	GNB
Lactose (MacConkey)	+	+ (mucoid)	-	-	-
Lactose (CLED)	+	+ (mucoid)	-	-	-
Oxidase	-	-	-	+	-
Catalase	+	+	+	+	+
Indole	+	- (K. pneumoniae)	+ (P. vulgaris)	-	-
Citrate	-	+	+ (P. vulgaris)	+	+
Urease	-	Weak +	Strong +	-	-
Motility	+	-	+ (swarming)	+	-

The principal differentiating features used for the identified Gram-negative bacteria are summarized in Table 2.

Table 2: Key differentiation features of the identified Gram-negative bacteria

E. coli	**Indole positive, lactose fermenter, motile**
Klebsiella	Mucoid, non-motile, lactose fermenter
Proteus	Swarming, urease
Pseudomonas	Oxidase positive, green pigment, non-lactose fermenter, motile
Acinetobacter	Oxidase negative, non-motile, non-lactose fermenter, cocco-bacilli

Antibiotic Sensitivity Testing

Phenotypic antimicrobial susceptibility testing was performed by the Kirby–Bauer disk-diffusion method on Mueller–Hinton agar according to CLSI guidance [11]. A suspension prepared from fresh pure colonies was adjusted to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. A uniform lawn was prepared by swabbing the agar surface in three directions, and antibiotic discs were applied within 15 minutes of inoculation. Plates were incubated aerobically at 37°C for 16–24 hours.

After incubation, inhibition-zone diameters were measured in millimetres and interpreted as susceptible, intermediate or resistant using the CLSI M100, 32nd edition breakpoints [11].

The tested antibiotics were amikacin (AK), meropenem (MP), cefoperazone/sulbactam (CFS), gentamicin (GM), tigecycline (TGC), nitrofurantoin (NIT), imipenem (IMP), colistin (CL), ceftazidime (CAZ), fosfomycin (FOS), norfloxacin (NX), piperacillin/tazobactam (PIT), ceftriaxone (CTR), ciprofloxacin (CIP), ofloxacin (OF) and aztreonam (AT). The organism-specific antibiotic panel is shown in Figure 1.

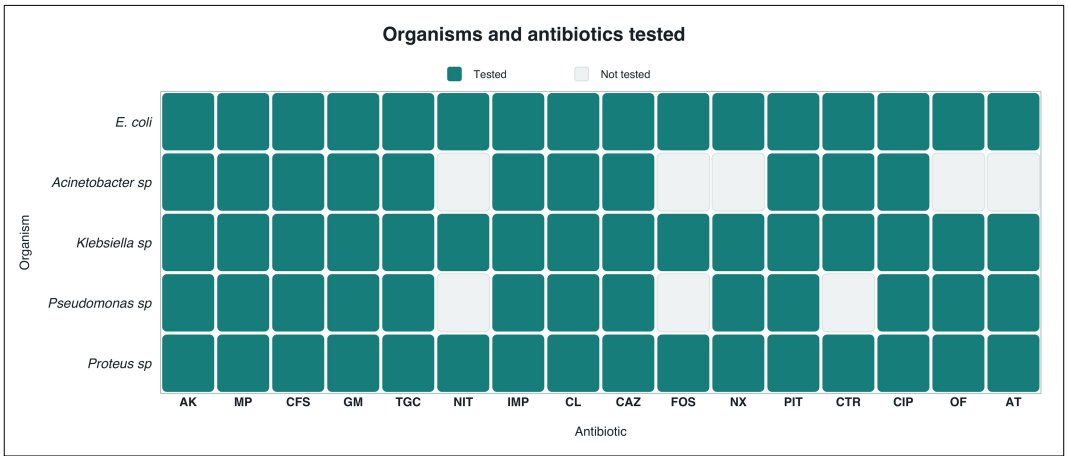

Figure 1: Antibiotic panel used for phenotypic antimicrobial susceptibility testing

VITEK 2 Compact Identification and AST

Ten isolates with prominent multidrug-resistant phenotypes were selected for automated identification and susceptibility testing using the VITEK® 2 Compact system [12].

Pure colonies from 24–48-hour nutrient agar cultures were suspended in 3 mL sterile saline. The suspension was mixed until homogeneous, and the turbidity was adjusted to 0.50–0.63 McFarland. Separate tubes prepared from the same suspension were used for the identification and AST cards.

The VITEK® 2 GN card was used for identification of Gram-negative bacteria, and the VITEK® 2 AST-N376 card was used for antimicrobial susceptibility testing.

Prepared cards were loaded into the instrument, sealed and incubated automatically for approximately 8–16 hours. Optical readings monitored biochemical reactions and bacterial growth, and the software compared the reaction profile with the system database to generate the organism identification.

For automated AST, the system measured growth in the presence of defined antimicrobial concentrations and calculated minimum inhibitory concentration values.

The software interpreted MIC results according to the applicable clinical breakpoints and generated the final organism identification and antimicrobial susceptibility report [12].

Data Analysis

Organism, sex, age and specimen distributions were summarized as counts and percentages. Resistance percentages were calculated using active denominators; results recorded as not tested, not applicable or missing were excluded. Putative multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial classes according to Magiorakos et al. [13]. Tabulation was performed in Microsoft Excel. Data processing and visualization were completed in RStudio [14] using ggplot2 [15], dplyr and tidyr through the tidyverse framework [16], and pheatmap for heatmap generation [17].

RESULTS

Sample Collection

A total of 440 bacterial isolates were collected from a diagnostic laboratory in Meerut. These isolates were received from different regional hospitals and clinics. Of these, 360 bacterial isolates were included for this study. The remaining isolates were excluded in case of missing information, no antibiotic susceptibility testing results or in case the isolates were from other than Gram negative bacteria. The remaining bacterial isolates came from a broad range of clinical samples including urine, sputum, pus swab, CSF etc. The largest sample group was urine with 200 isolates (55.6%). Pus samples formed the second largest group with 96 samples (26.7%). Blood and sputum contributed 19 and 17 isolates respectively while other sample types represented a smaller fraction (Figure 3).

The male-to-female distribution of the total sample was 165:195 with urine sharing the larger female part, with 135 female isolates and 65 male isolates. Pus, blood and sputum etc. showed a larger male share. This reflects a useful clinical background before looking at organism level distribution and resistance (Table 3).

Age Distribution

Most of the isolates belong to 19-40 years of age. This group consists of 132 isolates (36.7%) of the study population. The 41-60-year group followed with 103 isolates (28.6%). Patients above 60 and aged 18 or younger contributed 66 and 38 isolates, respectively. Though 21 isolates were missing the age details (Table 4).

Table 3: Organism and gender wise summary of sample collection

Sample type	Count	% of isolates	Male	Female	*E. coli*	*Klebsiella spp*	*Pseudomonas spp*	*Acinetobacter spp*	*Proteus spp*
Urine	200	55.6%	65	135	146	42	7	0	5
Pus	96	26.7%	56	40	56	18	22	0	0
Blood	19	5.3%	12	7	4	4	8	2	1
Sputum	17	4.7%	11	6	4	3	6	3	1
N/A	5	1.4%	3	2	3	1	1	0	0
ET tube	4	1.1%	3	1	0	3	0	1	0
Semen	4	1.1%	4	0	3	1	0	0	0
CSF	3	0.8%	2	1	0	0	2	1	0
Tube	2	0.6%	2	0	0	1	0	1	0
Bedsore	2	0.6%	2	0	1	0	1	0	0
Tissue	1	0.3%	1	0	0	0	1	0	0
Tracheostomy	1	0.3%	1	0	0	1	0	0	0
High vaginal swab	1	0.3%	0	1	1	0	0	0	0
Stent	1	0.3%	1	0	1	0	0	0	0
Peritoneal fluid	1	0.3%	1	0	1	0	0	0	0
Endocervical swab	1	0.3%	0	1	0	0	0	0	1
Tip	1	0.3%	0	1	1	0	0	0	0
ET aspiration	1	0.3%	1	0	**1**	**0**	**0**	**0**	**0**
Total	**360**	**100.0%**	**165**	**195**	**222**	**74**	**48**	**8**	**8**

Table 4: Age group distribution of clinical isolates.

Age group	Count	% of isolates
<=18	38	10.6%
19-40	132	36.7%
41-60	103	28.6%
>60	66	18.3%
Missing	21	5.8%

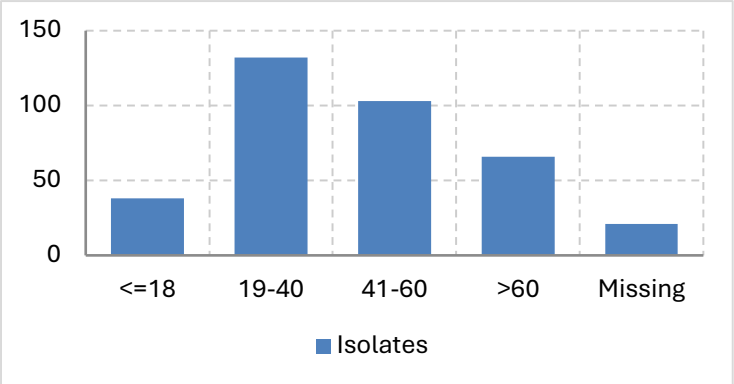

Figure 2: Age group distribution of the 360 bacterial isolates

Identification and Sex-wise Distribution of Organisms

The main population of the study isolates was dominated by *E. coli*. Which is the focus of this study. This organism accounted for 222 of 360 isolates which equals 61.7%. *Klebsiella spp* ranked second with 74 isolates, followed by *Pseudomonas spp* group 48 isolates. *Acinetobacter spp* and *Proteus spp* were identified in smaller numbers, with 8 isolates each (Figure 3).

A sex-wise pattern was also seen in organism distribution in the dataset. *E. coli* had female predominance, with 136 female isolates and 86 male isolates. *Klebsiella spp, Pseudomonas spp* and *Acinetobacter* were found more frequent in male patients. *Proteus spp* showed a female predominance with 5 isolates and 3 isolates in males (Table 5).

Table 5: Gender-wise Organism distribution

Organism	Count	% of isolates	Male	Female
E. coli	222	61.7%	86	136
Klebsiella spp	74	20.6%	44	30
Pseudomonas spp	48	13.3%	27	21
Acinetobacter spp	8	2.2%	5	3
Proteus spp	8	2.2%	3	5
Total	**360**	**100.0%**	**165**	**195**

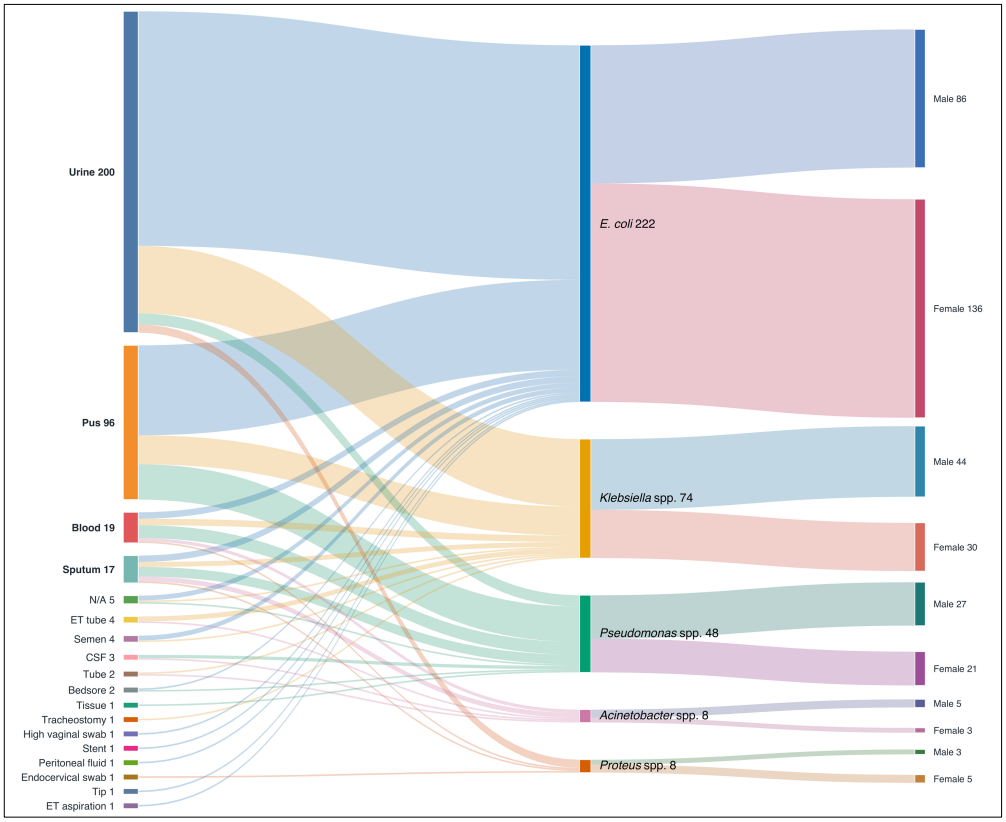

Figure 3: Sankey diagram showing the distribution of 360 isolates across sample types and bacterial organisms, with organism-specific male and female counts

Phenotypic Antibiotic Susceptibility Profile

Antibiotic resistance pattern varied across the tested drugs. 360 isolates showed different AST patterns against the tested antibiotics. An isolate level AST heatmap is given below which shows the resistance profiles of different organisms grouped together (Figure 4). Norfloxacin showed the highest resistance rate with 136 out of 184 tested isolates (73.9%). Ceftriaxone followed closely with resistance in 65 out of 92 tested isolates (70.7%). Other antibiotics showing high resistance were ciprofloxacin, ceftazidime and ofloxacin, each above 57%. The lowest resistance was seen in colistin, fosfomycin and tigecycline with 4.2%, 5.5%, 6.7%. Carbapenem resistance was lower than that of 3rd generation cephalosporin and fluoroquinolone, but it was still present. Meropenem and imipenem resistance observed was 22.2% and 23.0%, respectively. The antibiotic-adjuvant combination beta-lactam drugs showed lower resistance than ceftazidime and ceftriaxone alone. This difference became clearer when the resistance pattern was viewed by organism. The percentage calculation of AST data against each isolate was done based on the active denominator where antibiotic results shown as "not tested, N/A or missing" were excluded.

Table 6: Overall antibiotic resistance profile of the total isolates

Antibiotic	Class	Tested	Resistant	Resistant %
Norfloxacin NX	Fluoroquinolones	184	136	73.9%
Ceftriaxone CTR	Cephalosporins	92	65	70.7%
Ciprofloxacin CIP	Fluoroquinolones	154	90	58.4%
Ceftazidime CAZ	Cephalosporins	249	144	57.8%
Ofloxacin OF	Fluoroquinolones	125	72	57.6%
Aztreonam AT	Monobactams	116	55	47.4%
Gentamicin GM	Aminoglycosides	357	115	32.2%
Imipenem IM/IMP	Carbapenems	357	82	23.0%
Meropenem MP	Carbapenems	360	80	22.2%
Nitrofurantoin NIT	Nitrofuran	188	40	21.3%
Amikacin AK	Aminoglycosides	359	73	20.3%
Piperacillin Tazobactem PIT	Beta-lactam/BLI	358	70	19.6%
Cefoperazone Sulbactem CFS	Beta-lactam/BLI	332	55	16.6%
Tigecycline TGC	Glycylcycline	359	24	6.7%
Fosfomycin FO/FOS	Phosphonic acids	199	11	5.5%
Colistin CL	Polymyxins	355	15	4.2%

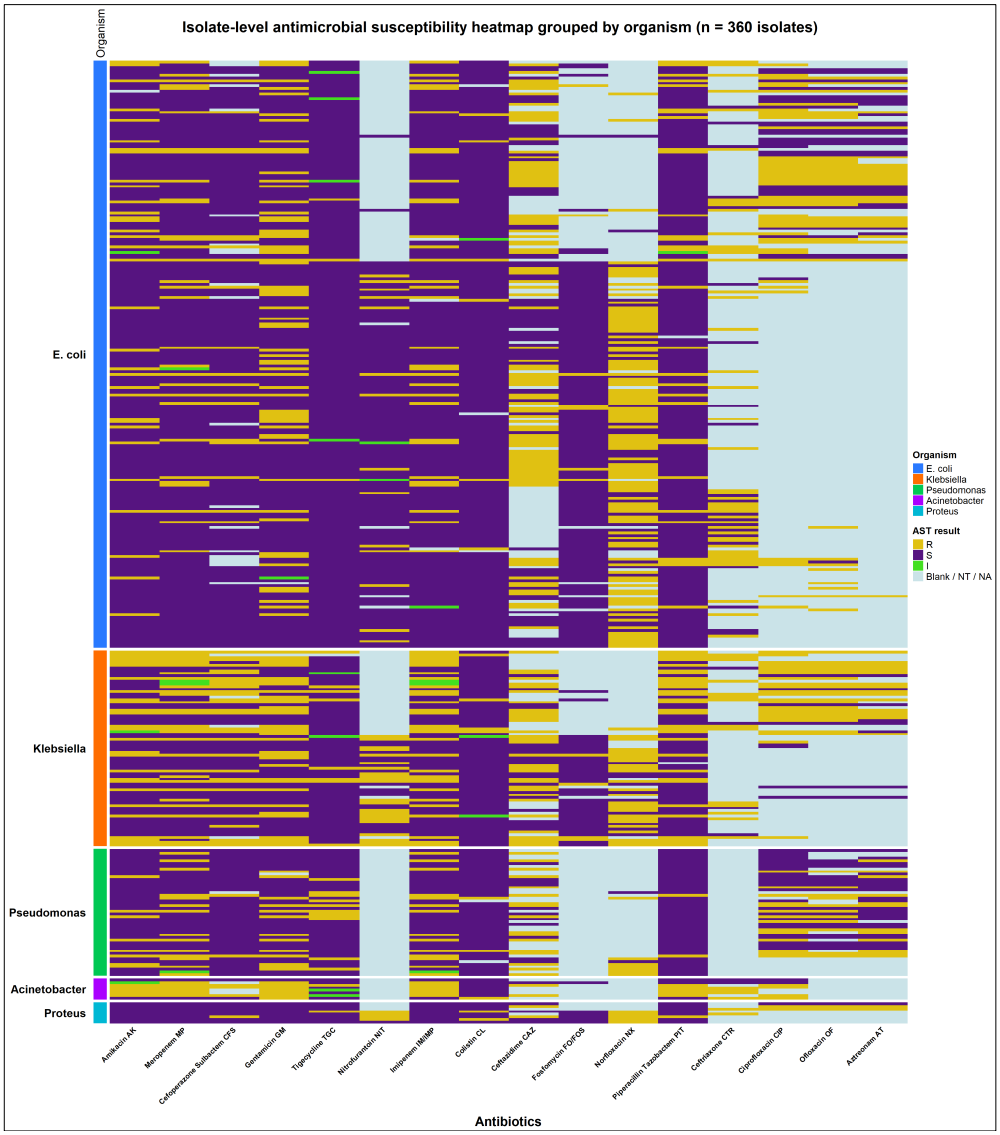

Figure 4: Isolate-level antimicrobial susceptibility heatmap grouped by organism in the full AST dataset

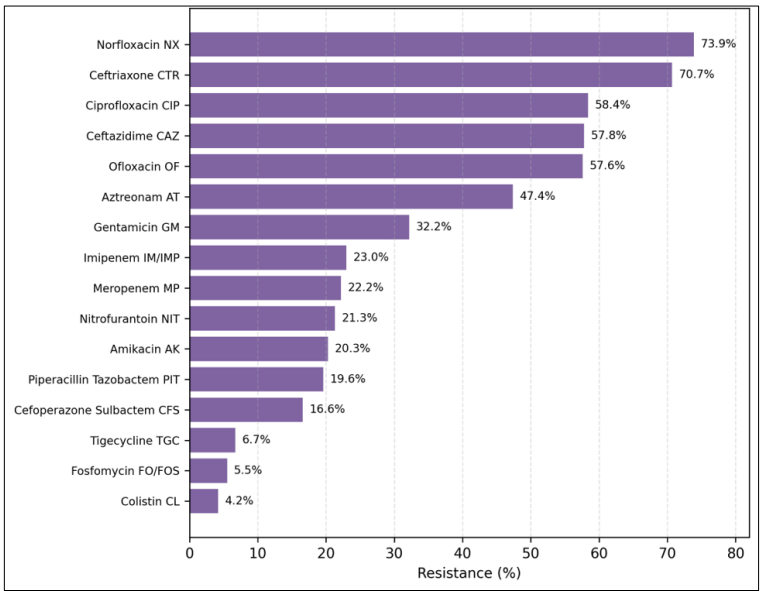

Figure 5: Overall resistance percentage for each antibiotic

Organism-wise Resistance Profile

The organism-wise resistance profile separated the resistance burden by bacterial group. *E. coli* showed the high resistance to ceftriaxone, ceftazidime, ciprofloxacin, ofloxacin and norfloxacin. On a better note, resistance to the last resort antibiotics like colistin, tigecycline remained low in *E. coli*. Fosfomycin, a primarily used antibiotic in urinary tract infection (UTI) came up with low resistance as well.

Klebsiella species showed a broader resistance profile than *E. coli* for several antibiotics such as ceftriaxone, norfloxacin, ciprofloxacin, ofloxacin and aztreonam. *Klebsiella* also showed higher resistance to amikacin, imipenem, meropenem and cefoperazone sulbactam compared to *E. coli*.

Pseudomonas species found resistant to ceftazidime and norfloxacin prominently, showing some lower values for some beta-lactam inhibitor combinations. *Acinetobacters* presented with 100% resistance to ceftazidime and fosfomycin in the tested subset. *Proteus* also showed 100% resistance to ceftriaxone and norfloxacin in the tested subset.

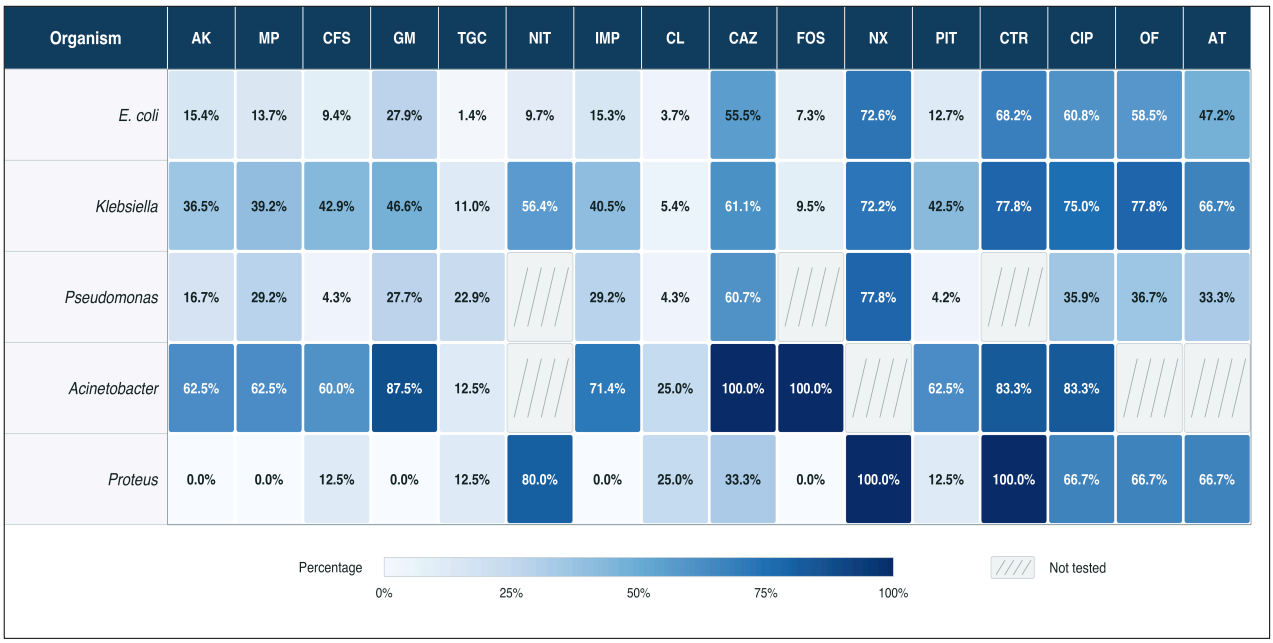

Figure 6: Tabular heatmap showing organism-wise resistance profile

Sample-wise Resistance Profile

The sample-wise resistance burden was carried out mainly in urine and pus isolates because they share the largest sample size of the dataset. Urine isolates showed high resistance to ceftriaxone, ceftazidime, ciprofloxacin, ofloxacin and norfloxacin. Pus isolates also showed a similar pattern with high resistance to ceftriaxone, ceftazidime, ciprofloxacin, ofloxacin and norfloxacin.

Sputum and blood isolates also showed important resistance patterns. Sputum isolates had high resistance to ceftriaxone, ceftazidime and norfloxacin, with raised resistance to gentamicin, ciprofloxacin and imipenem. Blood isolates also showed significant resistance to ceftriaxone and moderate resistance to gentamicin, ceftazidime, meropenem and fosfomycin. Several low-count sample types showed 100% resistance for some antibiotics which can be seen in the tabular heatmap below (Figure 7) but the urine, pus, blood and sputum isolates remained the main concern.

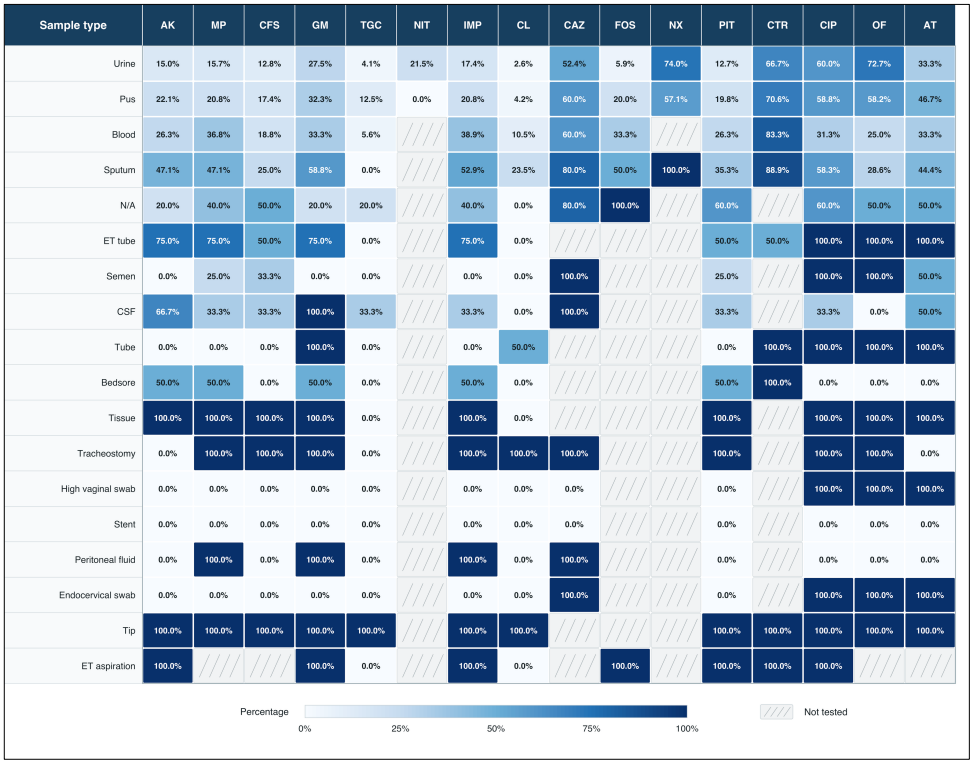

Figure 7: Tabular heatmap showing sample-wise resistance profile

Putative MDR Status of Organisms

Putative multi-drug resistance status of organisms was assessed based on the recommendation of Magiorakos et al, Isolates showing non-susceptibility to at least 1 antibiotic in not less than 3 antimicrobial classes were considered as MDR. Multi-drug resistance status differed across organisms. *Acinetobacter spp* and *Proteus spp* showed the highest MDR proportion with 6 of 8 isolates (75%). *Klebsiella spp* followed with 43 MDR isolates out of 74 (58.1%) [13].

Pseudomonas spp is comprised of 20 isolates with MDR status among 48 isolates (41.7%). *E. coli* had the largest absolute count of MDR isolates with 82 out 222 but its proportional MDR rate was lower at 36.9% which means *E. coli* dominated the isolated count, while Acinetobacter, Proteus and Klebsiella carried the stronger proportional MDR signals (Table 7 and Figure 8).

Table 7: Putative MDR status of the organisms

Organism	MDR count	Total	MDR %
E. coli	82	222	36.9%
Klebsiella spp	43	74	58.1%
Pseudomonas spp	20	48	41.7%
Acinetobacter spp	6	8	75.0%
Proteus spp	6	8	75.0%

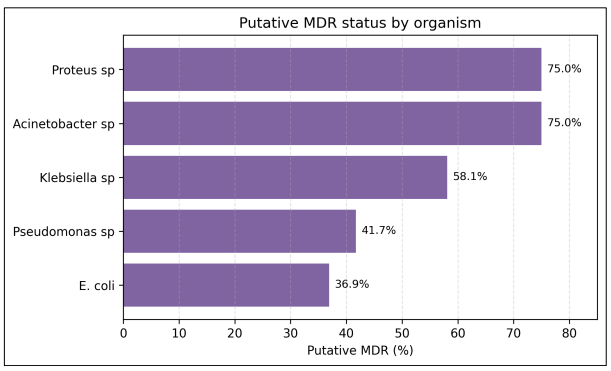

Figure 8: Distribution of putative MDR status of organisms

Overall Summary of Results

Antibiotic susceptibility testing shows three main findings. First, the dataset was mainly built from urine and pus isolates. Urine alone contributed more than half of all isolates. Second, *E. coli* was the dominant organism, followed by *Klebsiella spp* and *Pseudomonas spp*. Third, resistance was highest for fluoroquinolones and third generation cephalosporins, while colistin, fosfomycin and tigecycline showed lower overall resistance.

The MDR profile added a second layer to the same picture. *E. coli* contributed the largest number of MDR isolates because it was the dominant organism. However, *Acinetobacter spp, Proteus spp* and *Klebsiella spp* showed higher MDR proportions. This pattern supports organism-level reporting instead of pooled reporting alone.

DISCUSSION

The clinical collection was dominated by urine and pus, and *E. coli* represented 222 of 360 isolates (61.7%). The female predominance among *E. coli* isolates is compatible with its major role in urinary tract infection. The organism distribution also agrees with Indian hospital surveillance, where *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.* and *Acinetobacter spp.* remain major Gram-negative pathogens, although their relative proportions vary with specimen mix, patient population and level of care [18–21]. The high contribution of urine in this study should therefore be considered when interpreting the predominance of *E. coli*.

The *E. coli* profile showed substantial resistance to norfloxacin (72.6%), ceftriaxone (68.2%), ciprofloxacin (60.8%), ofloxacin (58.5%) and ceftazidime (55.5%). These findings fall within the wide Indian susceptibility ranges summarized by Veeraraghavan and Walia and show the same loss of activity of fluoroquinolones and third-generation cephalosporins [19]. In contrast, resistance in *E. coli* remained lower to meropenem (13.7%), imipenem (15.3%), piperacillin/tazobactam (12.7%), tigecycline (1.4%) and colistin (3.7%). This pattern indicates that several reserve agents retained activity, but the presence of carbapenem and colistin resistance remains clinically important.

Klebsiella spp. showed a broader resistance profile than *E. coli*, particularly for ceftriaxone (77.8%), ciprofloxacin (75.0%), ofloxacin (77.8%), aztreonam (66.7%), imipenem (40.5%) and meropenem (39.2%). Indian surveillance has reported carbapenem susceptibility of approximately 44–72% in *K. pneumoniae*, which corresponds to resistance levels that are comparable with the present findings [19]. The higher MDR proportion in *Klebsiella spp.* (58.1%) therefore supports organism-specific empirical policies rather than application of the pooled Gram-negative antibiogram to all Enterobacterales.

Pseudomonas spp. showed prominent resistance to norfloxacin (77.8%) and ceftazidime (60.7%), while resistance to imipenem and meropenem was 29.2% for each agent. These values are consistent with the wide inter-centre variation reported for Indian *P. aeruginosa*, where susceptibility to ceftazidime, carbapenems, fluoroquinolones and aminoglycosides differs markedly between hospitals [19]. *Acinetobacter spp.* showed 100% ceftazidime resistance and high imipenem and meropenem resistance, which agrees with the severe carbapenem-resistance burden reported for *Acinetobacter* in Indian surveillance and multicentre ICU datasets [19,21]. However, the *Acinetobacter* and *Proteus* groups contained only eight isolates each; their 75% MDR proportions and individual 100% resistance estimates should therefore be interpreted as strong local signals rather than stable prevalence estimates.

The present fluoroquinolone findings also agree with regional and global observations. The Asia-Pacific SMART study concluded that fluoroquinolones were unsuitable for empirical treatment of many urinary tract infections [22]. A later systematic review reported wide ciprofloxacin-resistance ranges, with particularly high estimates from India and neighbouring countries, while fosfomycin generally retained greater activity [23]. In the present dataset, total ciprofloxacin resistance was 58.4% and fosfomycin resistance was 5.5%; among *E. coli*, the corresponding values were 60.8% and 7.3%. These results support the same direction of resistance, while emphasizing that empirical treatment should follow local specimen- and organism-specific data.

The MDR pattern provides an additional comparison. *E. coli* contributed the largest absolute MDR count, but its proportional MDR rate was 36.9%. A GeoSentinel analysis across 57 international sites reported MDR in 37% of *E. coli* and 28% of *K. pneumoniae* infections [24]. The *E. coli* estimate is almost identical to the present result, whereas the local *Klebsiella spp.* MDR rate of 58.1% was considerably higher. The higher proportions in *Acinetobacter spp.* and *Proteus spp.* further show that pooled MDR counts alone can conceal an important organism-level burden.

Taken together, the phenotypic findings show sustained pressure on fluoroquinolones and third-generation cephalosporins, with a measurable but lower burden against carbapenems. The low overall resistance to colistin, fosfomycin and tigecycline should not be interpreted as support for unrestricted empirical use. These agents require careful organism-specific interpretation, appropriate susceptibility methods and antimicrobial stewardship. The agreement with Indian and international surveillance strengthens the need for continued local antibiogram development and AST-guided therapy [7,18,19].

This study was based on routine clinical laboratory isolates and does not estimate community prevalence.

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Antibiotics were not tested uniformly for every isolate; therefore, active denominators were used and direct comparison between drugs requires caution. The small numbers of *Acinetobacter spp.* and *Proteus spp.* produced unstable percentages. Patient-level information on admission status, ward, prior antimicrobial exposure, catheter use, recurrence, co-morbidity, clinical outcome and travel history was not available. These limitations restrict separation of community-acquired from healthcare-associated infection and prevent adjustment of resistance patterns for clinical risk factors.

CONCLUSION

The study used routine microbiology and antimicrobial susceptibility testing to define the phenotypic profile of clinical Gram-negative bacterial isolates, with main focus on *Escherichia coli*. *Escherichia coli* was the dominant organism among the included isolates. Urine and pus were the main sample sources. This finding supports the clinical importance of *Escherichia coli* in local urinary and wound-related infections.

The phenotypic resistance profile showed a clear burden of resistance against fluoroquinolones and third-generation cephalosporins. Norfloxacin and ceftriaxone showed the highest resistance signals in the total dataset. Ceftazidime, ciprofloxacin and ofloxacin also showed high resistance. Colistin, fosfomycin and tigecycline showed lower resistance. Carbapenem resistance was lower than cephalosporin and fluoroquinolone resistance, but it was still present. This pattern shows that common treatment options are under pressure in the local clinical setting.

The MDR findings show that absolute burden and proportional burden should be interpreted separately. *Escherichia coli* contributed the largest number of MDR isolates, while *Acinetobacter spp., Proteus spp.* and *Klebsiella spp*. showed higher MDR proportions. Routine phenotypic identification and AST therefore remain essential for organism-specific treatment decisions and local resistance surveillance. Continued monitoring of fluoroquinolone, third-generation cephalosporin and carbapenem resistance is required to support empirical-therapy policies and preserve the activity of the remaining effective antibiotics.

Author contributions

Mohd. Shadab: Investigation, data analysis, data curation, conceptualization, manuscript writing
Swati Tiwari: Sample acquisition, manuscript revision
Jyoti Saxena: Manuscript revision, manuscript writing
Dr. Poornima Shrivastava: Supervision, conceptualization, manuscript revision

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