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ANTIBIOTIC RESISTANCE PATTERN OF ESKAPE PATHOGENS AND MOLECULAR CHARACTERIZATION OF CARBAPENEMASE-PRODUCING PSEUDOMONAS AERUGINOSA IN VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE CENTER

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ABSTRACT

Background: The ESKAPE organisms cause a disproportionate share of healthcare-associated infection and escape commonly used antibacterial agents. Regional resistance data are sparse for newly established medical colleges in northern Tamil Nadu. The objective is to determine the prevalence and antibiotic resistance patterns of ESKAPE pathogens in clinical specimens, and to characterize carbapenemase production among Pseudomonas aeruginosa isolates by phenotypic and molecular methods.

Materials and Methods: A cross-sectional analytical study was carried out in a tertiary care teaching hospital over six months (November 2025 to April 2026). Consecutive, non-duplicate, clinically significant ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species) and Escherichia coli isolates from all clinical specimens were included. Susceptibility testing was by modified Kirby-Bauer disc diffusion and the VITEK 2 system, interpreted as per CLSI M100 (2025). Carbapenem-resistant P. aeruginosa isolates were tested by the modified carbapenem inactivation method (mCIM), the EDTA-modified carbapenem inactivation method (eCIM) and the meropenem-EDTA combined disc test and by real-time PCR for blaVIM, blaIMP and blaNDM. The chi-square test was used for associations.

Results: Of 458 isolates, Escherichia coli predominated (158, 34.5%), followed by Klebsiella species (95, 20.7%) and Enterococcus species (84, 18.3%). Urine was the commonest specimen (278, 60.7%). Multidrug resistance was found in 193 isolates (42.1%) and was commoner in isolates from male patients (48.3% versus 38.3%, P = 0.044). Third-generation cephalosporin resistance among Enterobacterales was 45.6%; methicillin resistance 33.3%; no vancomycin-resistant isolate was identified. Carbapenem resistance among P. aeruginosa was 10.4%; blaVIM was detected in all five isolates tested, with blaIMP and blaNDM not detected.

Conclusion: Multidrug resistance was widespread, while carbapenem resistance in P. aeruginosa remained comparatively low and no vancomycin resistance was detected. The uniform detection of the transferable metallo-beta-lactamase blaVIM nonetheless warrants active surveillance before it becomes entrenched locally.

Keywords: Drug Resistance, Multiple, Bacterial; Anti-Bacterial Agents; Carbapenems; Pseudomonas aeruginosa; Microbial Sensitivity Tests.

INTRODUCTION

Antimicrobial resistance has moved from a predominantly hospital-level inconvenience to a defining problem of contemporary clinical medicine. A systematic analysis of the global burden estimated that bacterial antimicrobial resistance was directly responsible for over one million deaths in 2019 and was associated with several million more, with the highest age-standardized mortality falling on countries of south Asia and sub-Saharan Africa.[1] India carries a substantial share of this burden, a consequence of high infectious disease incidence, widespread over-the-counter availability of antibacterial agents and uneven implementation of infection prevention practice.

The acronym ESKAPE was introduced to designate six organisms — Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species — that together account for the majority of healthcare-associated infections and that are able to "escape" the activity of commonly used antibacterial agents.[2-4] The group has since been widely used as a surveillance shorthand, and many authors now write ESKAPEE to include Escherichia coli, which shares the same resistance mechanisms, occupies the same clinical niches and is in most Indian laboratories the single commonest isolate.[5] The World Health Organization priority pathogens list, which guides global research and development, is populated almost entirely by members of this group.[6]

Among these organisms P. aeruginosa occupies a particular position. It is intrinsically resistant to several antibacterial classes, readily acquires further resistance determinants and is a frequent cause of ventilator-associated pneumonia, burn wound sepsis and infection of chronic wounds. Carbapenems have long been the mainstay of treatment for serious pseudomonal infection, and carbapenem-resistant P. aeruginosa (CRPA) therefore represents a therapeutic impasse associated with prolonged hospital stay and increased mortality. Carbapenem resistance in this species arises through several distinct mechanisms — loss or reduced expression of the OprD porin, upregulation of efflux systems such as MexAB-OprM, derepression of the chromosomal AmpC cephalosporinase, and acquisition of carbapenemase genes. Only the last of these is readily transmissible between organisms, and the metallo-beta-lactamases blaVIM, blaIMP and blaNDM are the variants most often reported from the Indian subcontinent.[7] Distinguishing carbapenemase-mediated resistance from the non-transmissible mechanisms matters clinically, because the former demands contact precautions and cohorting while the latter does not.

Antibiograms are strongly local. Resistance rates differ not only between countries but between hospitals within the same district, and empirical policy drawn from national aggregate data may be actively misleading in an individual institution. Government Medical College, Kallakurichi, is a recently established tertiary care teaching hospital serving a predominantly rural population in northern Tamil Nadu, and no published resistance surveillance data exist for this catchment area. The present study was undertaken to determine the prevalence and antibiotic resistance patterns of ESKAPEE pathogens isolated from clinical specimens at this center, and to characterize carbapenemase production among P. aeruginosa isolates by phenotypic and molecular methods, so as to provide a baseline for local empirical prescribing and antimicrobial stewardship.

MATERIALS AND METHODS

Study Design and Setting: This was a laboratory-based cross-sectional analytical study conducted in the Department of Microbiology, Government Medical College, Kallakurichi, Tamil Nadu, a tertiary care teaching hospital, over a period of six months from November 2025 to April 2026.

Study population and sample size: All clinical specimens — urine, pus and wound swabs, blood, endotracheal aspirates and sterile body fluids — received in the Microbiology laboratory during the study period were processed. The calculated sample size was 400 culture-positive isolates; consecutive sampling was continued to the end of the stipulated study period, yielding 458 isolates for analysis.

Inclusion and exclusion criteria: All clinically significant bacterial isolates identified as ESKAPE pathogens, together with E. coli, were included. Duplicate isolates of the same species from the same patient during the study period, environmental and surveillance samples, and isolates judged to represent colonization or contamination on clinico-microbiological correlation were excluded.

Specimen processing and identification: Specimens were processed according to standard microbiological techniques. Urine was inoculated by the semi-quantitative calibrated loop method on cystine lactose electrolyte deficient agar; other specimens were inoculated on 5% sheep blood agar and MacConkey agar, and blood was processed in an automated continuous-monitoring blood culture system with subculture of flagged bottles. Plates were incubated aerobically at 37 °C for 18 to 24 hours. Isolates were identified by colony morphology, Gram staining and conventional biochemical reactions, supplemented by the VITEK 2 automated identification system (bioMérieux, France) where required. Klebsiella, Acinetobacter and Enterococcus isolates that were not resolved to species level by the methods available were reported at genus level.

Antimicrobial susceptibility testing: Susceptibility testing was performed by the modified Kirby-Bauer

disc diffusion method on Mueller-Hinton agar and by the VITEK 2 automated system, and interpreted according to Clinical and Laboratory Standards Institute M100 (2025) breakpoints.[8] Antibiotic panels were selected according to the organism isolated and the representative site of infection as recommended by CLSI, so that not every agent was tested against every isolate; resistance rates are accordingly expressed as a proportion of isolates actually tested against each agent. Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 were used as control strains. Methicillin resistance in S. aureus was inferred from cefoxitin (30 µg) disc diffusion. Extended-spectrum beta-lactamase production was confirmed phenotypically by the combined disc test, comparing the zone of inhibition around a cefotaxime (30 µg) disc with that around a cefotaxime-clavulanic acid (30/10 µg) disc, an increase of 5 mm or more in the presence of clavulanic acid being taken as confirmatory- A disc diffusion plate is shown in Figure . Multidrug resistance was defined, following Magiorakos and colleagues, as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.[9]

Detection of carbapenemase production: Isolates of P. aeruginosa showing resistance to imipenem and/or meropenem on routine susceptibility testing were designated carbapenem resistant and taken up for further characterization. Carbapenemase production was screened by the modified carbapenem inactivation method (mCIM) and metallo-beta-lactamase production distinguished by the EDTA-modified carbapenem inactivation method (eCIM), performed and interpreted as described in CLSI M100 (2025); an increase of 5 mm or more in the eCIM zone diameter compared with the mCIM zone was taken as indicating a metallo-beta-lactamase.[8] Metallo-beta-lactamase production was additionally assessed by the combined disc test, in which the zone of inhibition around a meropenem (10 µg) disc was compared with that around a meropenem disc supplemented with 10 µL of 0.5 M EDTA; an increase of 7 mm or more in the presence of EDTA was interpreted as positive.

Molecular characterization: Carbapenem-resistant P. aeruginosa isolates were subjected to real-time polymerase chain reaction for the metallo-beta-lactamase genes blaVIM, blaIMP and blaNDM. Genomic DNA was extracted from 1 mL of an overnight broth culture using the PureFast® Bacterial DNA minispin purification kit (HELINI Biomolecules, Chennai, India) according to the manufacturer's instructions, comprising lysozyme digestion at 37 °C for 15 minutes, proteinase K treatment with binding buffer and an internal control template at 56 °C for 15 minutes, ethanol precipitation, and two spin-column wash steps followed by elution in 100 µL of elution buffer. The quality and quantity of extracted DNA were verified on a 1% agarose gel, and the purified DNA was stored at −20 °C until use.

Real-time PCR was performed using HELINI probe-based real-time PCR kits in a final reaction volume of 25 µL containing 10 µL of probe PCR master mix, 5 µL of primer-probe mix and 10 µL of purified DNA. A positive control supplied with the kit and a no-template control containing 10 µL of PCR-grade water were included in every run. Amplification was carried out with an initial Taq polymerase activation step at 95 °C for 15 minutes, followed by 35 cycles of denaturation at 95 °C for 20 seconds, annealing with data acquisition at 60 °C for 20 seconds and extension at 72 °C for 20 seconds. Fluorescence was detected in the FAM channel, with no passive reference dye and default ramping rate. A sample was reported as positive for the target gene when an exponential amplification curve crossed the threshold within 35 cycles.

Statistical analysis: Data were entered in Microsoft Excel and analyzed using standard statistical software. Categorical variables are presented as frequencies and percentages and continuous variables as mean and standard deviation. Associations between categorical variables were tested using the chi-square test, a P value below 0.05 being taken as statistically significant.

Ethical considerations: The study protocol was approved by the Institutional Ethics Committee, Government Medical College, Kallakurichi (Proposal No. GMCK/IEC/24/2025, dated October 27, 2025). As the study was laboratory based and used isolates obtained during routine diagnostic work-up without any additional intervention, a waiver of individual informed consent was granted. Patient identifiers were removed at the point of data entry and confidentiality was maintained throughout. The study was conducted in accordance with the Declaration of Helsinki and the National Ethical Guidelines for Biomedical and Health Research Involving Human Participants (2017), and is reported in accordance with the STROBE statement.[10]

RESULTS

Demographic and clinical profile: A total of 458 clinically significant, non-duplicate isolates were studied. Of these, 282 (61.6%) were recovered from female patients and 176 (38.4%) from male patients. The mean age of the patients was 37.6 ± 24.0 years, with a range extending from the neonatal period to the ninth decade. The largest single age band was 15 to 29 years, contributing 119 isolates (26.0%), followed by patients aged 60 years and above, who contributed 109 isolates (23.8%). Eighteen isolates (3.9%) were recovered from infants below one year of age. Urine was by far the commonest specimen, accounting for 278 isolates (60.7%), followed by pus and wound swabs with 128 (27.9%) and blood with 44 (9.6%); endotracheal aspirates and sterile

body fluids together contributed eight isolates (1.7%). General Medicine and General Surgery were the highest-yielding clinical units, contributing 201 (43.9%) and 138 (30.1%) isolates respectively, while the combined critical care areas — neonatal, pediatric, medical and surgical intensive care units — contributed 20 isolates (4.4%). The demographic and clinical profile is summarized in [Table 1].

Distribution of isolates: Escherichia coli was the predominant organism, accounting for 158 isolates (34.5%), followed by Klebsiella species with 95 (20.7%), Enterococcus species with 84 (18.3%), Pseudomonas aeruginosa with 48 (10.5%), Staphylococcus aureus with 36 (7.9%), Acinetobacter species with 31 (6.8%) and Enterobacter species with 6 (1.3%). All six ESKAPE genera were therefore represented, although Enterobacter was recovered only sporadically. Individual organisms showed distinct site preferences. Escherichia coli and Enterococcus species were overwhelmingly urinary, contributing 126 of 158 and 77 of 84 isolates from urine respectively, whereas P. aeruginosa was predominantly recovered from pus (35 of 48 isolates, 72.9%). Acinetobacter species showed the highest proportional representation in blood among the Gram-negative organisms, with 12 of 31 isolates (38.7%) from blood culture, and S. aureus likewise yielded 13 of 36 isolates (36.1%) from blood. The specimen-wise distribution is presented in [Table 2].

Resistance among Gram-negative isolates: Resistance patterns of the Gram-negative isolates are shown in Table 3. Among E. coli, resistance was high to ciprofloxacin (55.7%), cotrimoxazole (50.0%), amoxicillin-clavulanate (50.0%) and cefotaxime (48.7%), while piperacillin-tazobactam (3.8% resistance), amikacin (8.2%) and cefoperazone-sulbactam (12.0%) retained good activity. Nitrofurantoin, an important oral option for uncomplicated urinary infection, showed only 17.5% resistance among the 126 urinary E. coli isolates tested. Klebsiella species showed a broadly similar beta-lactam profile with 53.7% resistance to amoxicillin-clavulanate and 40.0% to cefotaxime, but markedly higher nitrofurantoin resistance at 54.4%. The six Enterobacter isolates were largely resistant to amoxicillin-clavulanate (five of six) and showed cefotaxime and cotrimoxazole resistance in three each, but no carbapenem resistance. On combined disc testing, 118 of 259 Enterobacterales isolates (45.6%) were confirmed as extended-spectrum beta-lactamase producers.

Acinetobacter species emerged as the most resistant organism in the series despite its modest numbers. Two-thirds of isolates (67.7%) were resistant to cefotaxime, and resistance reached 48.4% for amikacin, 38.7% for gentamicin, 35.5% for ceftazidime, 32.3% for piperacillin-tazobactam and cotrimoxazole, and 25.8% for both imipenem and meropenem. By contrast P. aeruginosa was comparatively susceptible: resistance was 31.2% to ceftazidime, 16.7% to piperacillin-tazobactam, 10.4% to imipenem, meropenem and ciprofloxacin, 8.3% to cefepime and 6.2% to amikacin.

Resistance among Gram-positive isolates: Twelve of 36 S. aureus isolates (33.3%) were cefoxitin resistant and therefore reported as methicillin-resistant S. aureus. Resistance among S. aureus was also high to erythromycin (58.3%), ciprofloxacin (58.3%), clindamycin (55.6%) and cotrimoxazole (38.9%), whereas tetracycline (2.8%) and doxycycline (5.6%) retained activity and no isolate was resistant to vancomycin or linezolid. Among the 84 Enterococcus isolates, 81.0% were ampicillin resistant and 64.3% ciprofloxacin resistant, while nitrofurantoin resistance was 29.9% among the 77 urinary isolates tested. No enterococcal isolate was resistant to vancomycin. Three isolates (3.6%) were reported as linezolid resistant and eight (9.5%) as teicoplanin resistant, all of which were vancomycin susceptible. Details are given in [Table 4].

Multidrug resistance: Applying the definition of non-susceptibility to at least one agent in three or more antimicrobial categories, 193 of 458 isolates (42.1%) were multidrug resistant. The highest organism-specific rate was in Enterobacter species at 66.7% (four of six), although this rests on very small numbers, followed by E. coli at 55.7% (88 of 158), S. aureus at 50.0%, Acinetobacter species at 41.9%, Klebsiella species at 36.8% and Enterococcus species at 34.5%. Pseudomonas aeruginosa had the lowest rate at 12.5% (six of 48). Multidrug resistance was significantly commoner among isolates from male patients than from female patients (48.3% versus 38.3%, P = 0.044). The apparent variation by specimen type — 44.5% in pus, 43.2% in urine and 29.5% in blood — did not reach statistical significance (P = 0.194), nor did the variation between clinical units (P = 0.553) or age bands (P = 0.141).

Carbapenem-resistant P. aeruginosa and carbapenemase genes: Five of the 48 P. aeruginosa isolates (10.4%) were resistant to both imipenem and meropenem and were classified as carbapenem-resistant P. aeruginosa. All five were recovered from pus, three from the Department of General Surgery and two from General Medicine, and all five were from adults aged 57 years and above. Three of the five were additionally resistant to ceftazidime and to piperacillin-tazobactam, and two of these were also resistant to cefepime, representing the isolates in the series resistant to all tested beta-lactam agents. All five remained susceptible to ciprofloxacin and amikacin. On phenotypic testing the carbapenem-resistant isolates were mCIM positive with a corresponding increase in zone diameter on eCIM, and showed zone enhancement around the meropenem-EDTA disc on combined disc testing, indicating metallo-beta-lactamase production.

All five carbapenem-resistant isolates were subjected to molecular characterization. Real-time PCR detected blaVIM in all five isolates tested (100%), while blaIMP and blaNDM were not detected in any isolate. Expressed against the full

series, blaVIM-positive P. aeruginosa accounted for five of 48 isolates of this species (10.4%) and five of 458 isolates overall (1.1%).

Table 1: Demographic and clinical profile of the study isolates (n = 458)

Characteristic	Number of isolates	Percentage
Age group (years)		
Below 1	18	3.9
1–14	65	14.2
15–29	119	26.0
30–44	70	15.3
45–59	77	16.8
60 and above	109	23.8
Sex		
Female	282	61.6
Male	176	38.4
Specimen type		
Urine	278	60.7
Pus / wound swab	128	27.9
Blood	44	9.6
Endotracheal aspirate	5	1.1
Sterile body fluid	3	0.7
Clinical unit		
General Medicine	201	43.9
General Surgery	138	30.1
Pediatrics	52	11.4
Obstetrics and Gynecology	44	9.6
Intensive care units (NICU, PICU, IMCU, SICU)	20	4.4
Others (Ophthalmology, ENT)	3	0.7

Mean age 37.6 ± 24.0 years. ENT: Ear, nose and throat; IMCU: Intensive medical care unit; NICU: Neonatal intensive care unit; PICU: Pediatric intensive care unit; SICU: Surgical intensive care unit.

Table 2: Specimen-wise distribution of the isolates (n = 458)

Organism	Urine	Pus	Blood	ET aspirate	Sterile body fluid	Total n (%)
Escherichia coli	126	28	4	0	0	158 (34.5)
Klebsiella species	57	30	5	2	1	95 (20.7)
Enterococcus species	77	3	4	0	0	84 (18.3)
Pseudomonas aeruginosa	5	35	6	0	2	48 (10.5)
Staphylococcus aureus	3	20	13	0	0	36 (7.9)
Acinetobacter species	8	8	12	3	0	31 (6.8)
Enterobacter species	2	4	0	0	0	6 (1.3)
Total	278	128	44	5	3	458 (100.0)

ET: Endotracheal.

Table 3: Antimicrobial resistance among Gram-negative isolates, expressed as number resistant / number tested (percentage)

Antimicrobial agent	Escherichia coli (n=158)	Klebsiella species (n=95)	Enterobacter species (n=6)	Pseudomonas aeruginosa (n=48)	Acinetobacter species (n=31)
Amoxicillin-clavulanate	79/158 (50.0)	51/95 (53.7)	5/6 (83.3)	NT	NT
Piperacillin-tazobactam	6/158 (3.8)	7/95 (7.4)	1/6 (16.7)	8/48 (16.7)	10/31 (32.3)
Cefoperazone-sulbactam	19/158 (12.0)	16/95 (16.8)	0/6 (0.0)	NT	NT
Cefotaxime	77/158 (48.7)	38/95 (40.0)	3/6 (50.0)	NT	21/31 (67.7)
Ceftazidime	NT	NT	NT	15/48 (31.2)	11/31 (35.5)
Cefepime	NT	NT	NT	4/48 (8.3)	8/31 (25.8)
Imipenem	5/51 (9.8)	6/33 (18.2)	NT	5/48 (10.4)	8/31 (25.8)
Meropenem	7/57 (12.3)	8/43 (18.6)	0/1 (0.0)	5/48 (10.4)	8/31 (25.8)
Amikacin	13/158 (8.2)	7/95 (7.4)	1/6 (16.7)	3/48 (6.2)	15/31 (48.4)
Gentamicin	45/158 (28.5)	9/95 (9.5)	1/6 (16.7)	NT	12/31 (38.7)
Ciprofloxacin	88/158 (55.7)	25/95 (26.3)	2/6 (33.3)	5/48 (10.4)	9/31 (29.0)
Cotrimoxazole	79/158 (50.0)	19/95 (20.0)	3/6 (50.0)	NT	10/31 (32.3)
Nitrofurantoin	22/126 (17.5)	31/57 (54.4)	1/2 (50.0)	NT	NT

NT: Not tested against this organism, in accordance with CLSI M100 (2025) organism-specific panels. Nitrofurantoin was tested on urinary isolates only.

Table 4: Antimicrobial resistance among Gram-positive isolates, expressed as number resistant / number tested (percentage)

Antimicrobial agent	Enterococcus species (n=84)	Staphylococcus aureus (n=36)
Ampicillin	68/84 (81.0)	NT
Cefoxitin	NT	12/36 (33.3)
Gentamicin	NT	5/36 (13.9)
Ciprofloxacin	54/84 (64.3)	21/36 (58.3)
Cotrimoxazole	NT	14/36 (38.9)
Nitrofurantoin	23/77 (29.9)	NT
Doxycycline	14/84 (16.7)	2/36 (5.6)
Tetracycline	NT	1/36 (2.8)
Erythromycin	6/84 (7.1)	21/36 (58.3)
Clindamycin	NT	20/36 (55.6)
Linezolid	3/84 (3.6)	0/36 (0.0)
Vancomycin	0/84 (0.0)	0/36 (0.0)
Teicoplanin	8/84 (9.5)	NT

NT: Not tested against this organism, in accordance with CLSI M100 (2025) organism-specific panels. Cefoxitin resistance in Staphylococcus aureus denotes methicillin resistance. Nitrofurantoin was tested on urinary isolates only.

Figure 1: Combined Disk test for ESBL detection (An increase of more than 5 mm zone of inhibition around a cefotaxime-clavulanic acid (30/10 µg) disc than cefotaxime (30 µg) disc on Mueller-Hinton agar- ADD this line. Discs shown are meropenem (MRP, 10 µg), piperacillin-tazobactam (PIT, 100/10 µg), ampicillin (AMP, 10 µg), cefaclor (CEC, 30 µg), cefotaxime (CTX, 30 µg), ciprofloxacin (CIP, 5 µg) and tetracycline (TE, 30 µg).

DISCUSSION

This study documents the resistance profile of 458 ESKAPEE isolates over a six-month period at a newly established tertiary care teaching hospital in northern Tamil Nadu. Two findings stand out. The first is the sheer breadth of multidrug resistance, present in more than four of every ten isolates. The second, and more encouraging, is that carbapenem resistance in P. aeruginosa remained low at 10.4%, in contrast to the picture from many established tertiary centers in India where rates well above 30% are routinely reported.[7] Taken together these observations suggest an institution in which resistance to older first-line agents is already entrenched in the community-acquired urinary flora, but in which the specifically hospital-adapted carbapenem-resistant phenotypes have not yet become established. That is precisely the situation in which stewardship and infection control investment yields the greatest return.

The predominance of E. coli, accounting for over a third of all isolates, mirrors the specimen mix, in which urine contributed three of every five samples, and is consistent with the pattern reported from other Indian centers in which ESKAPEE surveillance has been extended to include E. coli.[5,12] Klebsiella species collectively formed the second commonest group at 20.7%, and the scarcity of Enterobacter, which contributed only six isolates (1.3%), is noteworthy. This may reflect the true epidemiology of a hospital with a relatively short operational history and limited long-stay intensive care activity, since Enterobacter is characteristically an organism of prolonged hospitalization and prior broad-spectrum exposure. The comparatively low contribution of the intensive care units, at 4.4% of all isolates, supports this interpretation. It may equally reflect the limitations of conventional biochemical identification in resolving members of the Enterobacter cloacae complex, and the finding should be interpreted with that caveat.

The finding that 45.6% of Enterobacterales were confirmed extended-spectrum beta-lactamase producers is consistent with the high ESBL rates that have been reported across India over the past decade,[11] and reinforces the argument against empirical use of third-generation cephalosporins for serious infection at this center. Reassuringly, the beta-lactam-beta-lactamase inhibitor combinations performed well, with piperacillin-tazobactam resistance of only 3.8% in E. coli and 7.4% in Klebsiella species, and amikacin resistance below 10% in both. These agents, rather than the carbapenems, are the rational escalation option for most serious Gram-negative infection in this hospital at present. The low nitrofurantoin resistance among urinary E. coli is likewise clinically useful, and supports its continued use as first-line oral therapy for uncomplicated lower urinary tract infection, although the much higher rate in Klebsiella species means that it cannot be relied upon once a non-coli coliform has been identified. The most striking organism-specific finding is the

resistance profile of Acinetobacter species, which despite contributing only 6.8% of isolates was the most resistant organism in the series across almost every agent tested, with carbapenem resistance of 25.8%. Its disproportionate recovery from blood cultures and from critical care areas is characteristic of this genus and marks it as the principal target for infection control effort at this institution. The contrast with P. aeruginosa is instructive: the two organisms occupy overlapping hospital niches and are frequently grouped together in empirical prescribing policy, yet their local antibiograms diverge sharply, and a policy calibrated to Acinetobacter would substantially over-treat pseudomonal infection at this center.

The methicillin resistance rate of 33.3% among S. aureus falls within the range commonly reported from Indian tertiary centers and, in the absence of any vancomycin or linezolid resistance, indicates that these agents remain dependable for serious staphylococcal infection. Among enterococci, the complete absence of vancomycin resistance is a genuinely reassuring finding at an institution of this age and argues that the glycopeptides can still be relied upon for serious enterococcal infection here; it also establishes a clear baseline against which any future emergence of vancomycin-resistant enterococci can be recognized. The small number of isolates reported as linezolid or teicoplanin resistant while remaining vancomycin susceptible does not correspond to any well-characterized enterococcal resistance phenotype, and these results should be regarded as provisional pending confirmation by minimum inhibitory concentration testing. Ampicillin resistance in 81.0% of enterococci limits the value of this agent for empirical treatment of enterococcal urinary infection. Comparable ESKAPE surveillance from other settings — a multi-country meta-analysis from Africa and a five-year single-center series from south-eastern Romania — has likewise identified S. aureus and K. pneumoniae among the leading contributors, although the relative rank of individual species varies considerably with the specimen mix and case profile of the institution, which is precisely why locally generated antibiograms cannot be replaced by external data.[15,16]

The molecular findings, though based on a small number of isolates, are internally consistent and epidemiologically informative. All five carbapenem-resistant P. aeruginosa isolates carried blaVIM, and neither blaIMP nor blaNDM was detected; this accords with the metallo-beta-lactamase phenotype demonstrated on eCIM and on EDTA combined disc testing. Uniform carriage of a single carbapenemase gene among isolates recovered within a six-month window, the majority from a single clinical unit and all from pus, raises the possibility of a common source or of clonal spread within the surgical wards rather than independent acquisition. This distinction has direct operational consequences, and molecular typing of these isolates would be the logical next step. The predominance of blaVIM is consistent with the wider Indian picture, in which VIM and NDM are the metallo-beta-lactamases most often reported from P. aeruginosa, in contrast to the blaKPC-dominated epidemiology of North America and parts of Europe.[7,13] The clinical significance is that metallo-beta-lactamases are not inhibited by avibactam or vaborbactam, so the newer beta-lactam-beta-lactamase inhibitor combinations that have transformed the management of KPC-producing organisms offer no benefit here; cefiderocol and aztreonam-based combinations remain the theoretical options, neither of which is readily available at this center.

It is equally important to note what these findings do not establish. Carbapenem resistance in P. aeruginosa arises as often from porin loss, efflux upregulation and AmpC derepression as from acquired carbapenemases, and these chromosomal mechanisms are not transmissible.[14] The detection of a transferable metallo-beta-lactamase in every isolate tested is therefore the more concerning of the two possible results, since blaVIM is typically carried on integrons within mobile genetic elements and can disseminate to other Gram-negative species. Its presence at this center, even at low prevalence, argues for active surveillance of carbapenem-resistant Gram-negative isolates and for contact precautions and cohorting of affected patients, rather than for the reassurance that the low overall carbapenem resistance rate might otherwise invite.

Limitations: Several limitations should be borne in mind when interpreting these findings. The study was conducted at a single center over six months, so the results reflect local epidemiology and cannot be generalized. Antibiotic panels were selected according to organism and specimen site as recommended by CLSI rather than applied uniformly, so denominators differ between agents and organisms and some resistance rates rest on small numbers, most obviously for Enterobacter species. Klebsiella, Acinetobacter and Enterococcus isolates were not consistently resolved to species level, so the specific contributions of K. pneumoniae, A. baumannii and E. faecium — the organisms named in the ESKAPE acronym — cannot be separated from those of related species within each genus. Clinical outcome data were not collected, so the attributable impact of resistance on mortality and length of stay at this center could not be assessed. Finally, the number of carbapenem-resistant P. aeruginosa isolates available for molecular characterization was small, and the gene distribution observed should be regarded as descriptive rather than representative.

CONCLUSION

Multidrug resistance was present in 42.1% of ESKAPEE isolates at this center, with E. coli the commonest organism and Acinetobacter species the

most resistant. Nearly half of Enterobacterales were confirmed extended-spectrum beta-lactamase producers, so third-generation cephalosporins should no longer be relied upon for empirical treatment of serious infection here, while piperacillin-tazobactam, cefoperazone-sulbactam and amikacin retained good activity and represent the rational escalation options. Carbapenem resistance in P. aeruginosa was low at 10.4% and no vancomycin resistance was detected. This combination — entrenched resistance to older agents alongside preserved carbapenem and glycopeptide activity — defines a narrow and valuable window in which a structured antimicrobial stewardship program, coupled with strengthened infection prevention practice and periodic revision of the institutional antibiogram, can prevent the establishment of carbapenem-resistant organisms in this hospital.

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