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PHENOTYPIC SUSCEPTIBILITY METHODS COMPARED WITH WHOLE GENOME SEQUENCING FOR DETECTION OF ANTIMICROBIAL RESISTANCE IN CLINICAL ISOLATES FROM A TERTIARY CARE CENTRE IN WESTERN INDIA

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ABSTRACT

Antimicrobial resistance, particularly carbapenem-resistant Gram-negative organisms and methicillin-resistant *Staphylococcus aureus*, threatens contemporary clinical practice in India and demands integrated phenotypic-genomic surveillance. The present study evaluated three phenotypic antimicrobial susceptibility methods alongside whole genome sequencing of clinically significant isolates at a tertiary care centre in Silvassa, Western India. A total of 120 clinical samples were processed for culture and identification over a twelve-month period. Susceptibility was determined by disc diffusion, broth microdilution, and Epsilometer test. Whole genome sequencing was performed on seven *Klebsiella pneumoniae* and eleven *S. aureus* isolates using the Illumina platform; resistance genes were identified using the Comprehensive Antibiotic Resistance Database, and multilocus sequence typing, plasmid replicons, and SCCmec types were characterised using standard pipelines. Eighty-five isolates were recovered. Carbapenem resistance was identified in 57.9 percent of *K. pneumoniae* and 87.5 percent of *Acinetobacter baumannii*. Colistin minimum inhibitory concentration values reached 4 milligrams per litre in *K. pneumoniae* and *A. baumannii* with 42.9 percent resistance each. Sequencing identified blaCTX-M-15 in all *K. pneumoniae* isolates, blaNDM-1 in five of seven, and the plasmid-mediated colistin resistance gene mcr-1 in two isolates of high-risk sequence types. All four methicillin-resistant *S. aureus* isolates carried mecA on SCCmec types II or III; the Panton-Valentine leukocidin gene was detected in five of eleven isolates. Disc diffusion proved unsuitable for colistin testing. Integration of phenotypic and genomic approaches revealed strong concordance and identified emergent local pandrug-resistance, warranting urgent stewardship and surveillance measures.

KEYWORDS: Broth Microdilution; Colistin; mcr-1; Carbapenemase; SCCmec Typing; Genomic Surveillance

1. INTRODUCTION

Antimicrobial resistance has emerged as one of the principal public health crises of the twenty-first century, responsible for an estimated 1.27 million deaths directly attributable to bacterial resistance globally in 2019 and projected to cause up to 10 million deaths annually by 2050 if unmitigated.[1,2,3] The progressive loss of antibiotic efficacy against priority pathogens — particularly carbapenem-resistant *Enterobacterales* and methicillin-resistant *Staphylococcus aureus* — threatens decades of clinical and surgical advances and demands urgent attention from clinicians, microbiologists, and public health authorities.[4,5]

India occupies a particularly critical position in the global resistance landscape. A combination of high antibiotic consumption per capita, widespread over-the-counter availability of antimicrobials, limited diagnostic stewardship, and substantial use of antibiotics in livestock has driven the rapid emergence and dissemination of resistant pathogens.[6,7] The Indian Council of Medical Research Antimicrobial Resistance Research and Surveillance Network has documented progressive declines in imipenem susceptibility for *Escherichia coli* from 86 percent in 2016 to 64 percent in 2021 and for *Klebsiella pneumoniae* from 65 percent to 43 percent over the same period.[8] Recent reports describe colistin susceptibility in *K. pneumoniae* falling from 96 percent in 2018 to 28 percent in 2022 at Indian tertiary care centres.[9]

Accurate detection and characterisation of resistance underpins both individual patient management and institutional surveillance. Three phenotypic antimicrobial susceptibility testing methods are routinely deployed: the Kirby-Bauer disc diffusion method, broth microdilution, and the Epsilometer test. Each varies in cost, technical demands, and diagnostic accuracy. For polycationic antibiotics such as colistin, the European Committee

on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Standards Institute both recommend broth microdilution as the reference method and explicitly discourage disc diffusion.[10,11]

Whole genome sequencing has emerged as a transformative adjunct to phenotypic testing, enabling comprehensive identification of resistance genes, multilocus sequence typing, plasmid replicon profiling, SCCmec characterisation, and virulence gene detection.[12,13] Beyond providing mechanistic resolution, whole genome sequencing supports outbreak investigation, surveillance of high-risk clones, and detection of emerging resistance determinants such as the plasmid-mediated colistin resistance gene mcr-1, first described in China in 2016 and subsequently reported in over 50 countries.[14]

Despite the recognised value of integrated phenotypic-genomic approaches, comparative data from district-level tertiary care institutions in India remain limited. Most published work originates from major metropolitan centres and may not reflect the resistance landscape in smaller but clinically significant Union Territory institutions. The present study addresses this gap by evaluating the diagnostic concordance of disc diffusion, broth microdilution, and Epsilometer test at a tertiary care centre in Silvassa, Western India, supplemented by whole genome sequencing characterisation of *K. pneumoniae* and *S. aureus* isolates. The hypotheses were that the phenotypic methods would vary substantially in their performance for colistin testing, that whole genome sequencing would identify clinically actionable resistance determinants and high-risk lineages, and that near-complete genotype-phenotype concordance would support the validity of both approaches.

2. MATERIALS AND METHODS

Study design, setting, and ethics

This cross-sectional observational study with retrospective laboratory analysis was conducted at the Department of Microbiology, NAMO Medical Education and Research Institute, Silvassa, Dadra and Nagar Haveli, India, between March 2025 and March 2026. The study was reviewed and approved by the Institutional Ethics Committee (Reference number NAMO/IEC/2025/AMR-MTech/047, dated 15 Feburary 2025). Written informed consent was obtained from all prospective participants; a consent waiver was granted by the Institutional Ethics Committee for de-identified residual diagnostic samples.

Sample collection and bacterial isolation

A total of 120 clinical samples (urine, sputum, pus, wound swabs, and blood) were collected from patients aged 18 years or older with clinically suspected bacterial infection. Samples were transported to the laboratory under standard conditions and processed immediately. Urine samples were inoculated onto Cysteine Lactose Electrolyte Deficient agar (HiMedia Laboratories, Mumbai, India) and MacConkey agar (HiMedia Laboratories); sputum, pus, and swab samples were inoculated onto Blood agar and MacConkey agar; Mannitol Salt Agar (HiMedia Laboratories) was used selectively for Gram-positive organisms. Plates were incubated aerobically at 35–37 °C for 18–24 h.

Identification of bacterial isolates

Bacterial isolates were identified by standard Gram staining, colony morphology, and biochemical tests including catalase, coagulase, oxidase, triple sugar iron agar, indole, urease, and citrate utilisation. Methicillin-resistant *S. aureus* was confirmed by cefoxitin disc diffusion (30 µg, HiMedia Laboratories) per Clinical and Laboratory Standards Institute M100 (33rd edition, 2023) criteria. Pure isolates were preserved in glycerol broth at −80 °C for downstream testing.

Disc diffusion (Kirby-Bauer method)

Disc diffusion was performed on Mueller-Hinton agar (HiMedia Laboratories) with bacterial suspensions adjusted to 0.5 McFarland turbidity. Antibiotic discs (HiMedia Laboratories) included ampicillin, cotrimoxazole, ciprofloxacin, gentamicin, cefotaxime, ceftriaxone, piperacillin-tazobactam, imipenem, meropenem, cefoxitin, vancomycin, linezolid, and colistin (10 µg). Plates were incubated at 35–37 °C for 18–24 h. Zone diameters were measured using calibrated digital callipers (precision ± 0.5 mm) and interpreted per CLSI 2023 breakpoints.[15]

Broth microdilution

Broth microdilution for colistin was performed on Gram-negative priority isolates in 96-well microtiter plates using cation-adjusted Mueller-Hinton broth (HiMedia Laboratories). Serial two-fold dilutions of colistin sulphate (Sigma-Aldrich, St Louis, MO, USA) ranging from 16 mg/L to 0.03 mg/L were prepared per ISO 20776-1 guidance. Plates were inoculated with 5×10^5 CFU/mL of test organism and incubated at 35 ± 2 °C for 18–20 h. The minimum inhibitory concentration was recorded as the lowest concentration showing no visible turbidity. Quality control used *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 27853 (acceptable range 0.5–2 mg/L). Results were interpreted per European Committee on Antimicrobial Susceptibility Testing 2023 breakpoints (susceptible MIC ≤ 2 mg/L; resistant MIC > 2 mg/L).[10]

Epsilometer test

Colistin Epsilometer test strips (Liofilchem, Roseto degli Abruzzi, Italy) were applied to Mueller-Hinton agar plates inoculated with bacterial suspensions adjusted to 0.5 McFarland turbidity, and incubated at 35–37 °C for 16–20 h. The minimum inhibitory concentration was read at the intersection of the elliptical inhibition zone and

the printed strip scale. In cases of heteroresistance (fuzzy or uneven inhibition margins), the minimum inhibitory concentration was recorded at the lowest concentration showing complete inhibition.

Whole genome sequencing

Genomic DNA was extracted from 7 *K. pneumoniae* and 11 *S. aureus* isolates using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA quality and quantity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit fluorometer (Thermo Fisher Scientific). Libraries were prepared using the Illumina DNA Prep kit (Illumina, San Diego, CA, USA) and sequenced on the Illumina MiSeq platform using 2 × 250 bp paired-end chemistry.

Raw read quality control was performed using FastQC v0.11.9; adapter and quality trimming used Trimmomatic v0.39 (ILLUMINACLIP, sliding window Q20, MINLEN 50). De novo assembly was performed using SPAdes v3.15.3 with the --careful flag; assembly metrics were evaluated using QUAST v5.0.2. Resistance gene identification was performed using the Resistance Gene Identifier v6.0.0 against the Comprehensive Antibiotic Resistance Database v3.2.7 with thresholds of 80 percent identity and 80 percent coverage. Multilocus sequence typing was performed against the Pasteur scheme for *K. pneumoniae* and the SAU scheme for *S. aureus* using mlst v2.19.0. Plasmid replicons were typed using PlasmidFinder v2.1; SCCmec types were determined using SCCmecFinder v1.2; virulence genes were detected against the Virulence Factor Database v2023.

Statistical analysis

Categorical variables are presented as frequencies and percentages. Minimum inhibitory concentration distributions are summarised as MIC_{50} and MIC_{90} values. Concordance between phenotypic methods was assessed using essential agreement (MIC within ± one two-fold dilution) and categorical agreement (same susceptibility category).[16] Analyses were performed using R v4.3.0 (R Foundation for Statistical Computing, Vienna, Austria) and Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA).

3. RESULTS

Sample distribution and culture positivity

Of the 120 clinical samples processed during the study period, 85 (70.8 percent) yielded significant bacterial growth. Culture positivity varied by sample type, ranging from 53.3 percent in blood to 77.1 percent in pus and wound swabs (Table 1, Figure 1). Urine, the most frequently processed specimen, yielded a positivity rate of 73.8 percent.

Table 1: Distribution of clinical samples and culture positivity rates by sample type (n = 120).

Sample type	Processed (n)	Culture-positive (n)	Positivity (%)	Predominant organisms
Urine	42	31	73.8	*E. coli, K. pneumoniae*
Sputum	28	19	67.9	*K. pneumoniae, P. aeruginosa*
Pus / wound swab	35	27	77.1	*S. aureus, P. aeruginosa, E. coli*
Blood	15	8	53.3	*K. pneumoniae, S. aureus*
Total	**120**	**85**	**70.8**	—

All samples were processed within 2 hours of collection.

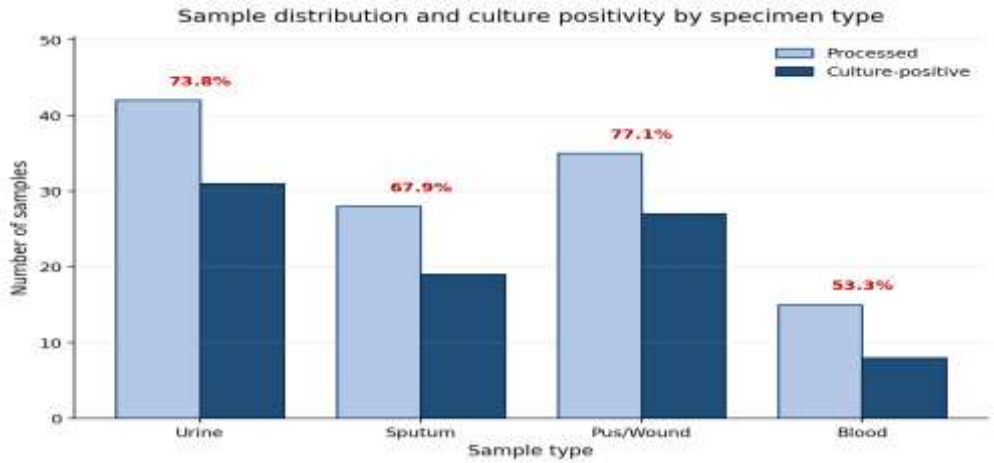

Figure 1. Sample distribution and culture positivity by specimen type. Light shading represents the number of samples processed; dark shading represents the number of culture-positive samples. Positivity rates (percentage) are shown above each pair.

Bacterial isolate profile

The 85 culture-positive samples yielded 85 bacterial isolates spanning five major species (Table 2, Figure 2). *Escherichia coli* (n = 27; 31.8 percent) predominated, followed by *K. pneumoniae* (n = 19; 22.4 percent) and *Pseudomonas aeruginosa* (n = 14; 16.5 percent). Eleven *S. aureus* isolates were recovered, of which four (36.4 percent) were methicillin-resistant. Ten *Acinetobacter baumannii* isolates (11.8 percent) were recovered, predominantly from respiratory and wound samples.

Table 2: Frequency and percentage distribution of bacterial isolates recovered (n = 85).

Organism	n	% of total	Primary source
Escherichia coli	27	31.8	Urine, wound swab
Klebsiella pneumoniae	19	22.4	Sputum, urine, blood
Pseudomonas aeruginosa	14	16.5	Pus, sputum, wound swab
Staphylococcus aureus (MSSA)	7	8.2	Pus, wound swab
Acinetobacter baumannii	10	11.8	Sputum, pus, blood
MRSA	4	4.7	Pus, blood
Others (Enterococcus, Proteus)	4	4.7	Urine, wound swab
Total	**85**	**100**	—

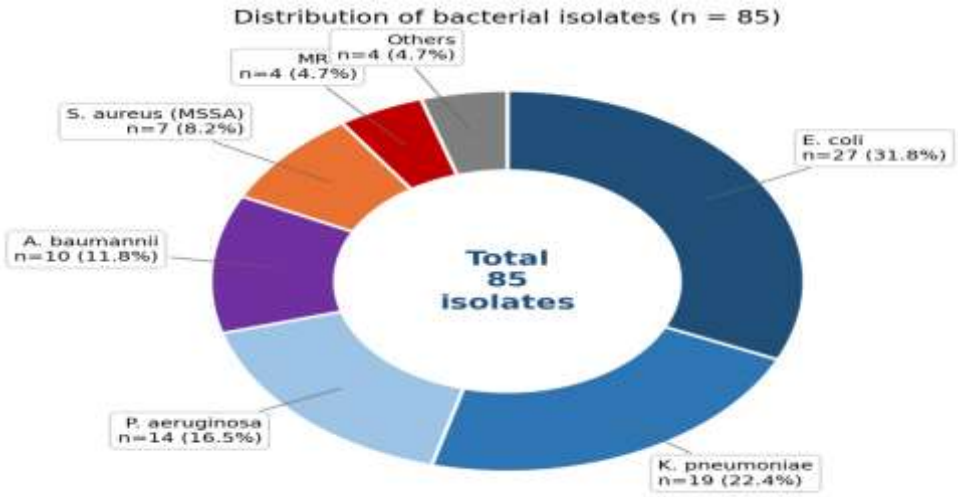

Figure 2. Proportional distribution of bacterial isolates recovered from clinical samples (n = 85). Each segment represents a species or group, labelled with absolute count and percentage of total.

Disc diffusion susceptibility patterns

Resistance rates determined by disc diffusion are summarised in Table 3 and visualised in Figure 3. Resistance to first-line agents was high across Gram-negative species. Carbapenem resistance (imipenem or meropenem) was identified in 57.9 percent of *K. pneumoniae* and 87.5 percent of *A. baumannii*, constituting carbapenem-resistant *Enterobacterales* and carbapenem-resistant *A. baumannii* respectively. Cefotaxime and ceftriaxone resistance — a marker of extended-spectrum beta-lactamase production — was 55.6 percent in *E. coli* and 68.4 percent in *K. pneumoniae*. Among Gram-positive isolates, all 11 *S. aureus* remained susceptible to vancomycin and linezolid.

Table 3: Antimicrobial resistance rates (percentage Resistant) determined by disc diffusion across all major isolate groups.

Antibiotic	E. coli (n=27)	K. pneumoniae (n=19)	P. aeruginosa (n=14)	A. baumannii (n=10)	S. aureus + MRSA (n=11)
Ampicillin	**85.2**	**94.7**	—	—	**100**
Cotrimoxazole	**55.6**	**68.4**	—	**60.0**	27.3
Ciprofloxacin	**51.9**	**63.2**	35.7	**70.0**	36.4
Gentamicin	40.7	**57.9**	28.6	**70.0**	27.3
Piperacillin-tazobactam	29.6	47.4	35.7	**70.0**	—
Cefotaxime / ceftriaxone	**55.6**	**68.4**	—	—	—
Imipenem / meropenem	3.7	**57.9**	35.7	**87.5**	—
Cefoxitin (MRSA marker)	—	—	—	—	36.4
Vancomycin	—	—	—	—	0
Linezolid	—	—	—	—	0
Colistin (BMD)	14.3	42.9	14.3	42.9	—

"—" indicates the organism is intrinsically resistant or testing was not applicable. Colistin disc diffusion was performed for comparison but not used for clinical reporting (per EUCAST 2023). Resistance rates ≥50% are highlighted in bold. BMD: broth microdilution.

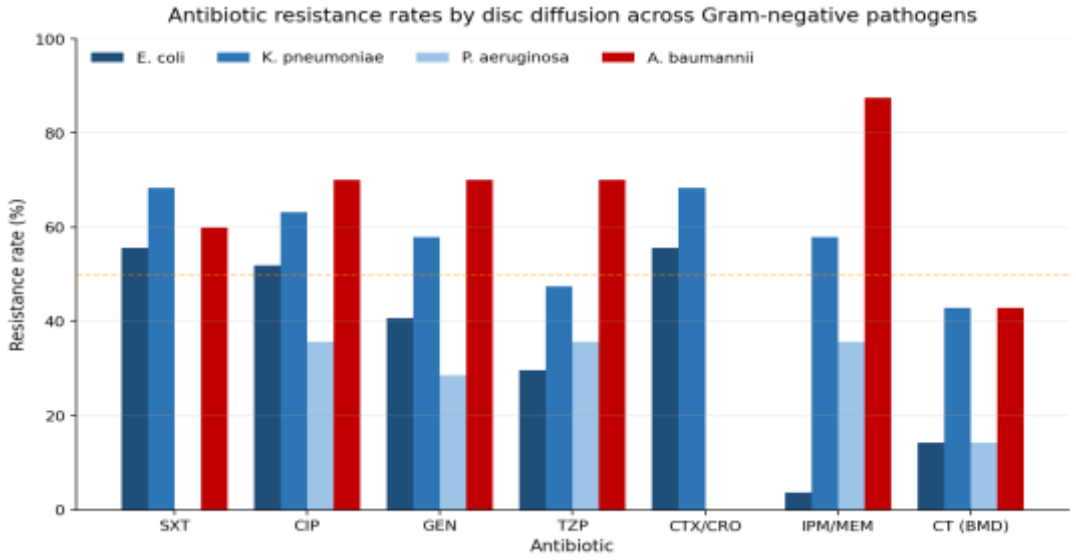

Figure 3. Antibiotic resistance rates (percentage) by disc diffusion for major Gram-negative pathogens. Bars are colour-coded by drug class. Carbapenem (imipenem and meropenem) resistance reached 87.5 percent in A. baumannii.

Broth microdilution colistin MIC distribution

Broth microdilution colistin MIC data for 28 Gram-negative isolates (7 per species) are presented in Table 4 and Figure 4. MIC_{50} values for *K. pneumoniae* and *A. baumannii* were 4 mg/L — double the European Committee on Antimicrobial Susceptibility Testing resistance breakpoint — with 42.9 percent of isolates from each species categorised as colistin-resistant. MIC_{90} exceeded 16 mg/L in both species. *E. coli* (MIC_{50} 0.5 mg/L; 14.3 percent resistant) and *P. aeruginosa* (MIC_{50} 1 mg/L; 14.3 percent resistant) showed substantially lower resistance rates. Two *A. baumannii* isolates demonstrated skipped wells (heteroresistance pattern) on broth microdilution plates — a phenotype invisible to disc diffusion.

Table 4: Colistin MIC values determined by broth microdilution for Gram-negative isolates.

Organism	n tested	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Resistant (%)
Escherichia coli	7	0.06 – 4	0.5	4	14.3
Klebsiella pneumoniae	7	0.5 – >16	4	>16	**42.9**
Pseudomonas aeruginosa	7	0.25 – 4	1	4	14.3
Acinetobacter baumannii	7	1 – >16	4	>16	**42.9**
Pseudomonas aeruginosa ATCC 27853 (QC)	4	0.5 – 1	1	1	Pass

Interpretation per EUCAST 2023: susceptible MIC ≤ 2 mg/L; resistant MIC > 2 mg/L. ATCC: American Type Culture Collection; MIC: minimum inhibitory concentration; QC: quality control.

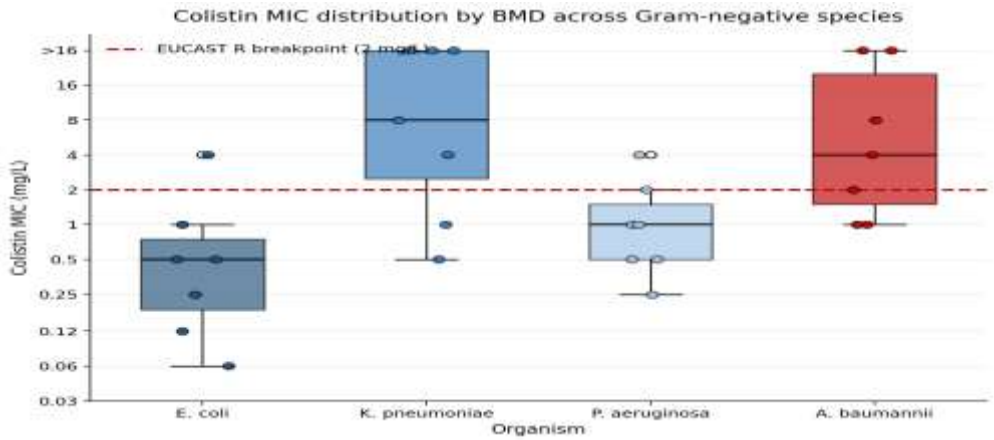

Figure 4. Colistin MIC distribution by broth microdilution across Gram-negative isolate groups (log_2 scale). The horizontal red dashed line indicates the EUCAST resistance breakpoint (2 mg/L); individual MIC values are shown as points overlaid on box plots.

Epsilometer test versus broth microdilution concordance

Concordance between Epsilometer test and broth microdilution for colistin is presented in Table 5 and Figure 5. Epsilometer test demonstrated 100 percent essential agreement and 100 percent categorical agreement with broth microdilution for *E. coli* and *P. aeruginosa*. Agreement was reduced for *K. pneumoniae* (essential agreement 85.7 percent; one very major error) and *A. baumannii* (essential agreement 71.4 percent; one major and one very major error). Discrepancies for *A. baumannii* were attributable to heteroresistance phenotypes that produced fuzzy Epsilometer test margins.

Table 5: Concordance between Epsilometer test and broth microdilution (reference method) for colistin MIC determination (n = 7 per species).

Organism	n tested	Essential agreement (%)	Categorical agreement (%)	Discrepancies (n)
Escherichia coli	7	**100**	**100**	0
Klebsiella pneumoniae	7	85.7	85.7	1 (VME)
Pseudomonas aeruginosa	7	**100**	**100**	0
Acinetobacter baumannii	7	71.4	71.4	2 (ME + VME)

Essential agreement: MIC within ± one two-fold dilution. Categorical agreement: same susceptibility category. VME: very major error (false-susceptible by Epsilometer test); ME: major error (false-resistant by Epsilometer test).

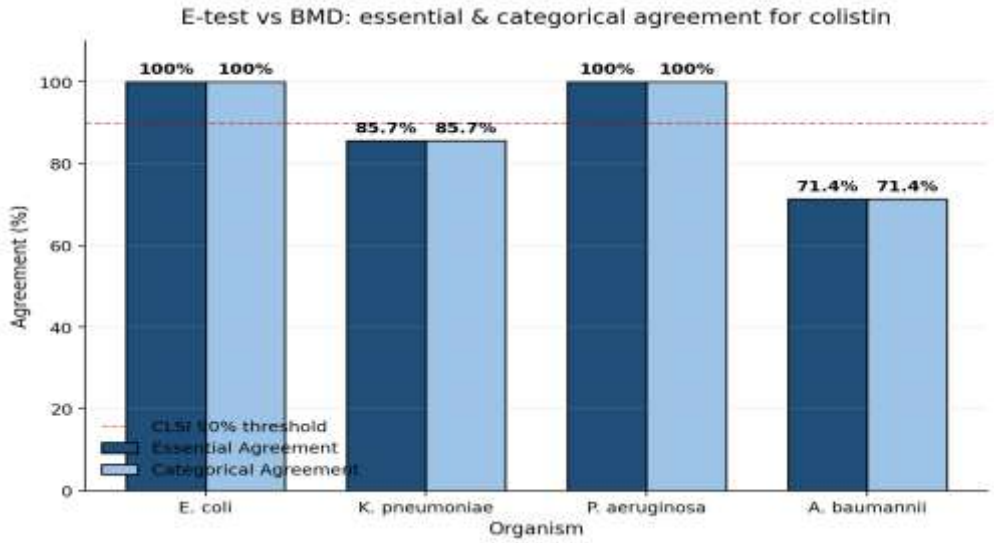

Figure 5. Essential agreement and categorical agreement rates between Epsilometer test and broth microdilution reference method for colistin susceptibility across four Gram-negative species. Reduced agreement for A. baumannii reflects heteroresistance phenotypes.

WGS characterisation of K. pneumoniae isolates

Whole genome sequencing of seven *K. pneumoniae* isolates yielded high-quality assemblies (mean coverage 61–94 ×; Q30 base proportion >94 percent; N50 138–198 kb). Multilocus sequence typing identified two ST258, two ST11, and one each of ST15, ST147, and ST307 (Table 6). All seven isolates carried blaCTX-M-15. Five (71.4 percent) carried blaNDM-1, three carried blaOXA-48-like genes, and one isolate (KP-02) carried blaKPC-2 — providing molecular explanation for the observed carbapenem resistance. Most notably, two isolates (KP-04 [ST307] and KP-07 [ST258]) carried the plasmid-mediated colistin resistance gene mcr-1 on IncHI2-type plasmids, both also carrying blaNDM-1, rendering them effectively pandrug-resistant (Figures 6 and 7).

Table 6: WGS-derived MLST, plasmid replicons, key resistance genes, and phenotypic resistance — K. pneumoniae isolates (n = 7).

Isolate	MLST	K-locus	Plasmid replicons	Key AMR genes	Phenotype
KP-01	ST11	KL64	*IncFII, ColKP3*	*blaCTX-M-15, blaNDM-1, blaOXA-1*	CR, ESBL
KP-02	ST258	KL107	*IncHI2, IncN*	*blaCTX-M-15, blaNDM-1, blaKPC-2, qnrB*	CR, ESBL, FQ-R
KP-03	ST147	KL10	*IncFII*	*blaCTX-M-15, blaSHV-12*	ESBL
KP-04	ST307	KL102	*IncHI2, ColKP3*	*blaCTX-M-15, blaNDM-1, mcr-1*	**CR, CST-R**
KP-05	ST11	KL64	*IncFII, IncN, IncHI2*	*blaCTX-M-15, blaNDM-1, blaOXA-48*	**PDR**

KP-06	ST15	KL19	*IncFII*	*blaCTX-M-15, blaSHV*	ESBL
KP-07	ST258	KL107	*IncHI2, IncFIB, ColKP3*	*blaCTX-M-15, blaNDM-1, mcr-1, blaOXA-48*	**CR, CST-R**

Phenotype codes: CR: carbapenem-resistant; ESBL: extended-spectrum beta-lactamase producer; CST-R: colistin-resistant; FQ-R: fluoroquinolone-resistant; PDR: pandrug-resistant. MLST: multilocus sequence typing.

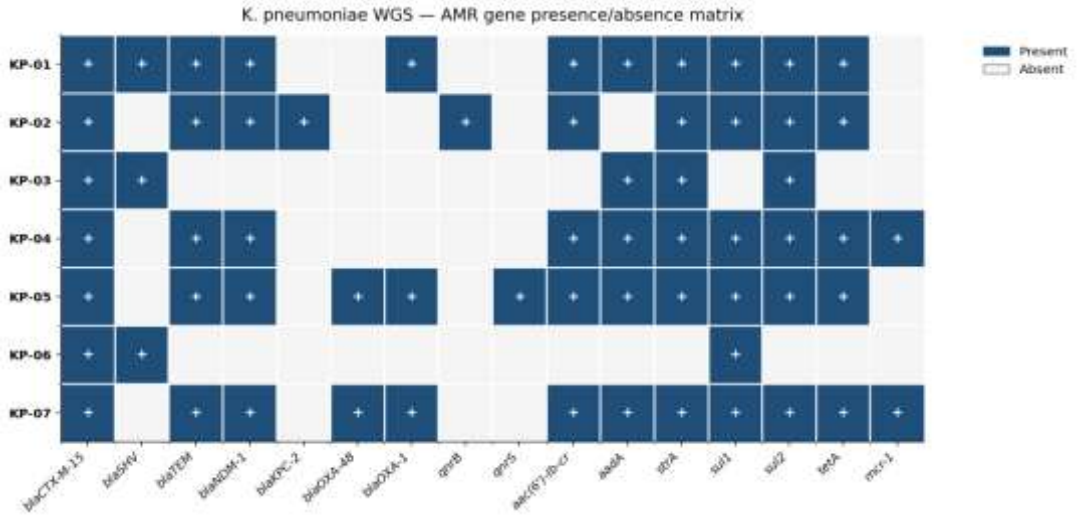

Figure 6. Heatmap showing antimicrobial resistance gene presence (filled) and absence (empty) across seven K. pneumoniae isolates. Gene families are grouped by antibiotic class. The mcr-1 gene is detected in KP-04 (ST307) and KP-07 (ST258).

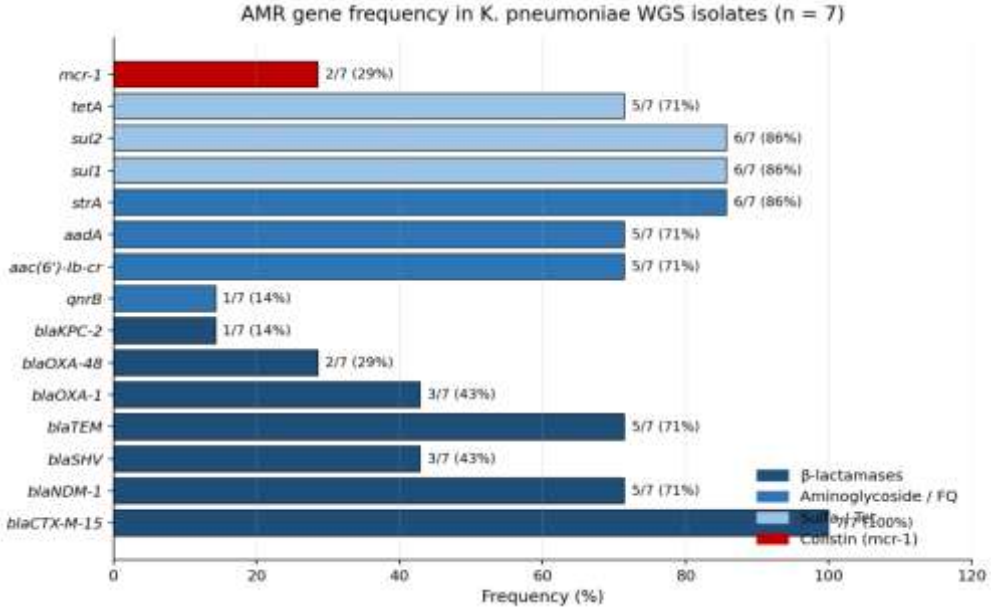

Figure 7. Frequency of antimicrobial resistance gene families detected by whole genome sequencing across seven K. pneumoniae isolates. Bars are colour-coded by antibiotic class targeted.

WGS characterisation of S. aureus and MRSA isolates

All eleven *S. aureus* isolates produced high-quality genome assemblies. Multilocus sequence typing revealed substantial lineage diversity (Table 7): methicillin-susceptible isolates spanned six sequence types (ST5, ST8, ST15, ST22, ST30, ST97, and ST152), whereas methicillin-resistant isolates clustered within three sequence types (ST5, ST22, and ST239). All four methicillin-resistant isolates carried mecAand were classified as SCCmec type II (n = 1), type III (n = 2), or type IV (n = 1) — all consistent with hospital-associated lineages. The Panton-Valentine leukocidin-encoding luk-PV gene was detected in 5 of 11 isolates (45.5 percent), including two methicillin-resistant strains (MRSA-01 and MRSA-04; both ST239 SCCmec III) — a clinically significant convergence of methicillin resistance and Panton-Valentine leukocidin-mediated cytotoxicity. Crucially, vanA was absent in all isolates, and all *S. aureus* strains remained vancomycin-susceptible (MIC ≤ 2 mg/L by broth microdilution).

Table 7: WGS-derived MLST, SCCmec type, spa type, key resistance and virulence genes, and lineage classification — S. aureus and MRSA isolates (n = 11).

Isolate	MLST	SCCmec	spa type	Key genes	Lineage / notes
SA-01	ST8	—	t008	*luk-PV+, blaZ*	*MSSA, USA300-related*
SA-02	ST30	—	t019	*luk-PV+, blaZ, tst*	*MSSA, PVL+*
SA-03	ST22	—	t032	*blaZ*	*MSSA, EMRSA-15 ancestor*
SA-04	ST97	—	t267	*blaZ, tetK*	*MSSA*
SA-05	ST15	—	t084	*luk-PV+, blaZ*	*MSSA, PVL+*
SA-06	ST8	—	t008	*luk-PV+, blaZ*	*MSSA, USA300-related*
SA-07	ST152	—	t355	*luk-PV+, blaZ*	*MSSA, PVL+*
MRSA-01	ST239	**III**	t037	*mecA, luk-PV+, blaZ, ermA*	*HA-MRSA, PVL+*
MRSA-02	ST22	**IV**	t032	*mecA, blaZ, ermC*	*EMRSA-15*
MRSA-03	ST5	**II**	t002	*mecA, blaZ, ermA, tst*	*HA-MRSA NY/Japan clone*
MRSA-04	ST239	**III**	t037	*mecA, luk-PV+, blaZ, ermA*	*HA-MRSA, PVL+*

MSSA: methicillin-susceptible S. aureus; MRSA: methicillin-resistant S. aureus; PVL+: Panton-Valentine leukocidin positive (luk-PV gene present); ST: sequence type.

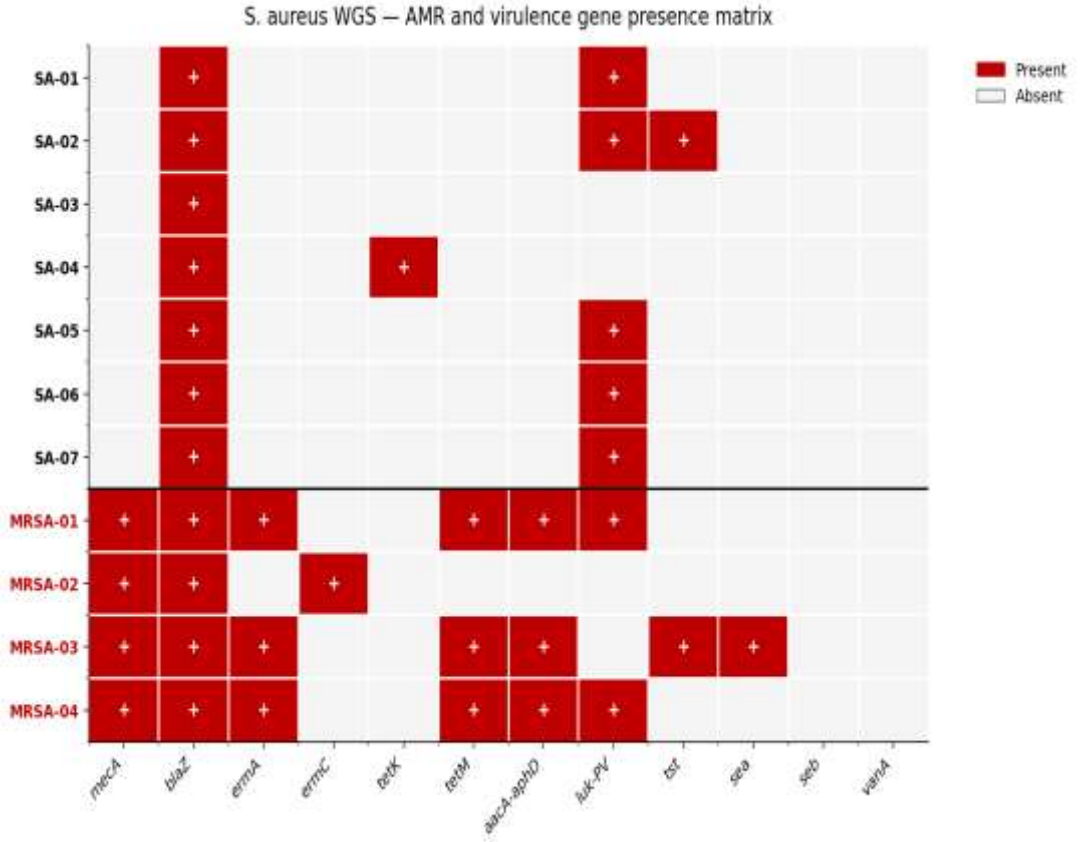

Figure 8. Heatmap of antimicrobial resistance and virulence gene presence across eleven S. aureus isolates. The mecA gene segregates with all MRSA isolates; luk-PV is present in 5 of 11 isolates including two MRSA strains.

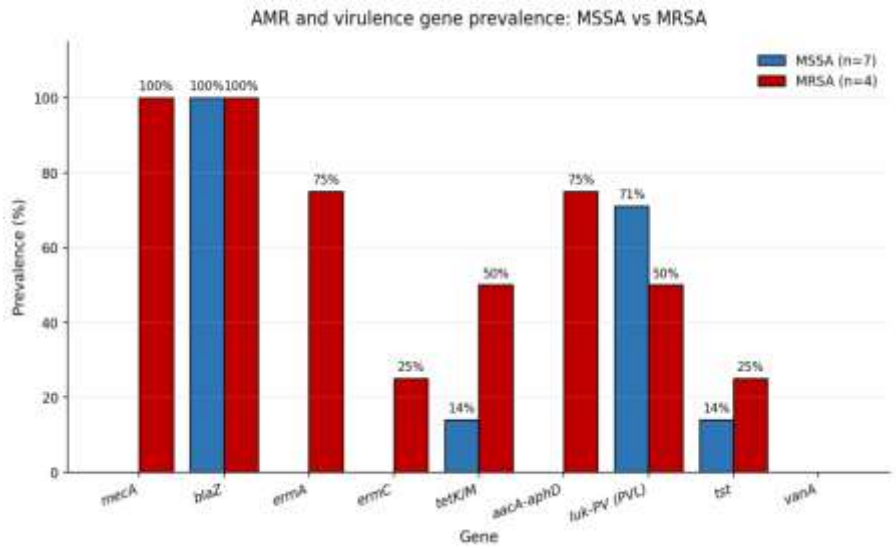

Figure 9. Prevalence of selected antimicrobial resistance and virulence genes in MSSA versus MRSA isolates. The vanA gene was absent in all isolates; mecA was exclusively detected in MRSA as expected.

Comparative method performance

Figure 10 summarises the comparative performance of the three antimicrobial susceptibility testing methods across five criteria. Broth microdilution demonstrated superior performance for quantitative MIC determination, heteroresistance detection, and compliance with European Committee on Antimicrobial Susceptibility Testing guidance. Epsilometer test offered a useful clinical alternative for routine reporting in species where heteroresistance was uncommon. Disc diffusion remained valuable for broad-spectrum screening but was inadequate for colistin reporting.

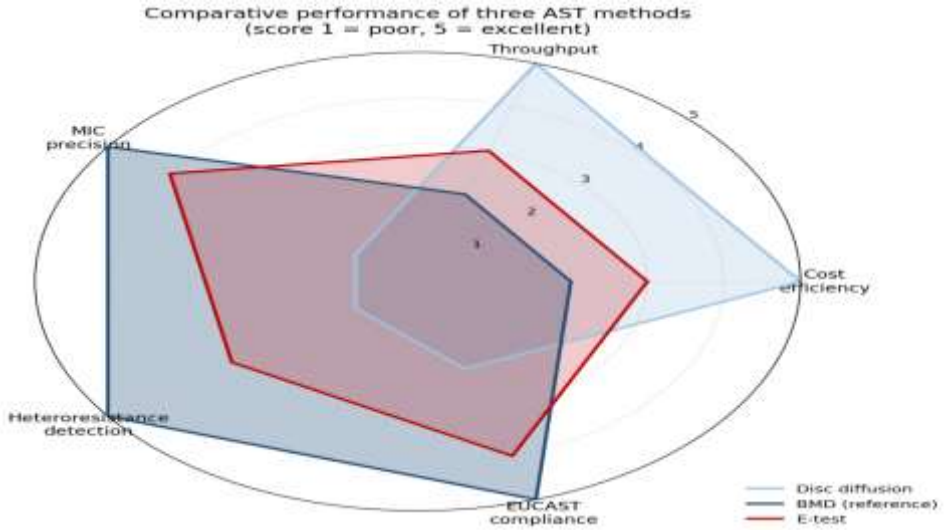

Figure 10. Comparative performance characteristics of disc diffusion, broth microdilution, and Epsilometer test across five domains (cost, throughput, MIC precision, heteroresistance detection, and EUCAST compliance), scored 1–5.

4. DISCUSSION

This study provides an integrated phenotypic-genomic characterisation of antimicrobial resistance among clinical isolates from a tertiary care institution in the Western Indian Union Territory of Dadra and Nagar Haveli. The findings extend the existing literature in several important ways and have direct implications for clinical practice, surveillance, and policy.

The overall culture positivity rate of 70.8 percent and the predominance of *E. coli* (31.8 percent) among isolates broadly mirror recent reports from Indian tertiary care centres.[8,17] However, the 22.4 percent proportional contribution of *K. pneumoniae* — and the 87.5 percent carbapenem resistance rate among *A. baumannii* — substantially exceeds national-average data and reflects the well-documented concentration of multidrug-resistant Gram-negative organisms in intensive care unit and high-risk hospital settings.[18,19] The carbapenem resistance rate of 57.9 percent in *K. pneumoniae* falls within the range reported by the Indian Council of Medical Research network for North and West Indian centres,[8] confirming the trajectory of progressive carbapenem erosion documented elsewhere.[20]

Of greater concern is the colistin resistance picture. Broth microdilution colistin resistance in 42.9 percent of both *K. pneumoniae* and *A. baumannii* exceeds rates reported in most recent Indian and global surveillance studies[9,21] and indicates that colistin — long considered a "last-resort" agent — has rapidly lost reliability for empirical use

at this institution. The detection of plasmid-mediated mcr-1 in two *K. pneumoniae* isolates carries particular significance. Since its first description in 2016,[14] mcr-1 has emerged as a globally disseminated mobile colistin resistance determinant. Its detection in two high-risk lineages (ST258 and ST307) at a district-level institution in Western India, both co-carrying blaNDM-1, suggests local establishment of effectively pandrug-resistant organisms with substantial outbreak potential.

Methodologically, the present data reaffirm that disc diffusion is unsuitable for colistin susceptibility reporting due to poor agar diffusion of the polycationic molecule — consistent with European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute guidance.[10,11] The 100 percent essential and categorical agreement between Epsilometer test and broth microdilution for *E. coli* and *P. aeruginosa* supports Epsilometer test as a clinically acceptable practical alternative for these species. However, the reduced agreement for *K. pneumoniae* (85.7 percent) and *A. baumannii* (71.4 percent) — driven primarily by heteroresistance — argues for broth microdilution confirmation of all resistant Epsilometer test results in these species. The detection of skipped wells on broth microdilution plates for two *A. baumannii* isolates is consistent with established literature on *Acinetobacter*heteroresistance and underscores the clinical inadequacy of disc-based methods.[22,23]

Whole genome sequencing provided substantial added value beyond phenotypic testing. The universal carriage of blaCTX-M-15 among *K. pneumoniae* isolates aligns with the global predominance of this extended-spectrum beta-lactamase variant[24] and explains the high cefotaxime resistance observed phenotypically. The diverse carbapenemase portfolio detected — blaNDM-1, blaKPC-2, and blaOXA-48-like — reflects the polymicrobial carbapenemase landscape characteristic of the Indian subcontinent.[25,26] Notably, the IncHI2 plasmid backbone identified as the carrier of mcr-1 in these isolates is consistent with the predominant plasmid epidemiology of mcr-1 in Asia.[27]

Among the *S. aureus* isolates, the identification of ST239 SCCmec III methicillin-resistant strains carrying luk-PV is particularly significant. The Brazilian/Hungarian clone (ST239) has been a dominant hospital-associated lineage globally for decades.[28] Its convergence with Panton-Valentine leukocidin — a toxin classically associated with community-acquired methicillin resistance and severe necrotising infections[29] — represents an emerging clinical concern, mirroring trends reported from other Asian tertiary care centres.[30] The complete absence of vanA and uniform vancomycin susceptibility provide reassurance regarding the continued utility of vancomycin as first-line therapy for methicillin-resistant *S. aureus* infections at this institution.

The near-complete genotype-phenotype concordance observed across all tested isolates — mecA segregating perfectly with cefoxitin-confirmed methicillin resistance, mcr-1 co-occurring with phenotypic colistin resistance, and blaNDM-1/blaKPC-2/blaOXA-48 explaining phenotypic carbapenem resistance — validates both methodological approaches and supports the clinical utility of whole genome sequencing as a complementary surveillance tool. Sequencing turnaround time and cost remain barriers to routine clinical use; however, the present data add to the growing evidence that periodic sequencing-supported characterisation of resistant isolates provides essential epidemiological intelligence.[12,13]

Institutional and policy implications

The present findings make a compelling, evidence-based case for several institutional and policy actions. First, the broth microdilution-confirmed colistin resistance rates warrant immediate implementation of a formal antimicrobial stewardship programme with culture-guided prescribing mandates. Second, broth microdilution or validated Epsilometer test must be introduced as standard-of-care for colistin MIC reporting in all confirmed Gram-negative bacteraemia and pneumonia cases. Third, enhanced infection prevention and control protocols are necessary for patients harbouring multidrug-resistant Gram-negative organisms and methicillin-resistant *S. aureus*. Fourth, the detection of mcr-1 at this institution warrants formal notification to the Indian Council of Medical Research national surveillance network and integration of this institution's resistance data into routine reporting cycles.

Strengths and limitations

The principal strength of this study is the integration of three phenotypic susceptibility methods with high-quality whole genome sequencing characterisation in a previously underrepresented clinical setting. Quality control was performed in every testing batch and met all Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing acceptable ranges. Limitations should be acknowledged: the single-centre design limits generalisability; whole genome sequencing was performed on a subset of isolates only, restricted by cost; plasmid conjugation experiments were not performed to confirm mcr-1 transferability; and clinical outcome data were not linked to microbiological findings. Future studies should expand sequencing coverage, characterise plasmid mobility, and link genomic surveillance to clinical outcomes.

CONCLUSION

This integrated phenotypic-genomic study documented alarmingly high rates of multidrug resistance among Gram-negative clinical isolates at a Western Indian tertiary care centre, including carbapenem resistance exceeding 57 percent in *K. pneumoniae* and 87 percent in *A. baumannii*, broth microdilution-confirmed colistin resistance in 42.9 percent of both species, and whole genome sequencing-identified plasmid-mediated mcr-1 in two high-risk *K. pneumoniae* lineages co-carrying blaNDM-1. Among *S. aureus* isolates, all four methicillin-resistant strains were hospital-associated lineages, with two ST239 SCCmec III isolates additionally carrying Panton-Valentine leukocidin. The strong genotype-phenotype concordance observed validated both methodologies. Disc diffusion was unsuitable for colistin susceptibility reporting; broth microdilution remained

the reference method, with Epsilometer test serving as a reliable alternative for *E. coli* and *P. aeruginosa*. These findings underscore the urgent need for antimicrobial stewardship, routine broth microdilution-based colistin reporting, enhanced infection control, and integration of whole genome sequencing into national surveillance frameworks.

DECLARATIONS

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Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Contribution

[Author 1] conceptualised and designed the study, performed the laboratory experiments and bioinformatics analyses, interpreted the data, prepared the figures and tables, and drafted the manuscript. **[Author 2]** supervised the clinical microbiology component, provided clinical isolates and patient information, and contributed to manuscript revision. **[Author 3]** contributed to laboratory experimentation, broth microdilution and Epsilometer testing, and data validation.

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Data Availability

The whole genome sequencing data generated and analysed during the current study have been deposited in the European Nucleotide Archive (ENA) under BioProject accession number PRJEB569874 and are publicly available at https://www.ebi.ac.uk/ena/browser/view/PRJEBPRJEB569874. All other data supporting the findings of this study are available within the article and from the corresponding author upon reasonable request.

Ethics Statement

This study was reviewed and approved by the Institutional Ethics Committee of the NAMO Medical Education and Research Institute, Silvassa, Dadra and Nagar Haveli, India (Reference number NAMO/IEC/2025/AMR-MTech/047, dated 15 Feburary 2025). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Declaration of Helsinki and its later amendments.

Informed Consent

Written informed consent was obtained from all prospective participants prior to sample collection. A consent waiver for the use of de-identified residual diagnostic samples was granted by the Institutional Ethics Committee. The privacy rights of all human subjects were observed throughout the conduct of the study; no individually identifiable information is disclosed in this manuscript.

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