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GENOTYPIC AND PHENOTYPIC CORRELATION OF MULTIDRUG-RESISTANT PATHOGENS ISOLATED FROM POST-SURGICAL WOUND INFECTIONS AT A TERTIARY CARE INSTITUTE

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ABSTRACT

Background: Surgical site infection (SSI) is among the commonest healthcare-associated infections in Indian tertiary care hospitals, and the progressive accumulation of transferable resistance determinants in SSI pathogens has narrowed empirical treatment options. Phenotypic susceptibility testing remains the operational basis of therapy in most Indian laboratories, yet the extent to which it reflects the underlying resistance genotype in post-surgical wound isolates is not well characterised in the western Indian setting.

Objectives: To determine the bacteriological profile and antimicrobial resistance pattern of pathogens isolated from post-surgical wound infections, to characterise the principal β-lactam resistance determinants by polymerase chain reaction (PCR), and to quantify the agreement between phenotypic and genotypic detection of methicillin resistance, extended-spectrum β-lactamase (ESBL) production and carbapenemase production.

Methods: A prospective, cross-sectional, observational study was conducted in the Department of Microbiology in collaboration with the Department of Surgery at NAMO Medical Education and Research Institute, Silvassa, from July 2025 to July 2026. Patients undergoing surgical procedures were monitored for SSI using CDC/NHSN case definitions. Isolates were identified and tested for antimicrobial susceptibility by automated broth microdilution and Kirby–Bauer disc diffusion, interpreted per CLSI M100. Phenotypic screening comprised the cefoxitin disc test, the combined-disc ESBL confirmatory test and the modified carbapenem inactivation method (mCIM/eCIM). All isolates underwent multiplex and singleplex PCR for mecA, blaCTX-M, blaTEM, blaSHV, blaNDM, blaOXA-48, blaKPC, blaVIM, blaIMP, blaOXA-23 and blaOXA-51. Agreement was expressed as Cohen's κ with 95% confidence intervals; associations were tested by Pearson's χ^2 test.

Results: Of 2,184 surgical procedures monitored, 178 met the case definition for SSI (8.15%; 95% CI 7.05–9.37). Cultures were positive in 156 cases (87.6%), yielding 187 non-duplicate isolates; 29 infections (18.6%) were polymicrobial. Gram-negative bacilli accounted for 135 isolates (72.2%), led by Klebsiella pneumoniae (38; 20.3%) and Escherichia coli (34; 18.2%); Staphylococcus aureus was the commonest Gram-positive isolate (33; 17.6%). Overall, 117 isolates (62.6%) were multidrug-resistant. Cefoxitin resistance was present in 15 of 33 S. aureus (45.5%) and mecA in 16 (48.5%). Among 88 Enterobacterales, 54 (61.4%) were phenotypic ESBL producers and 56 (63.6%) carried an ESBL determinant, blaCTX-M predominating (47; 53.4%); 27 (30.7%) were carbapenem non-susceptible and 28 (31.8%) carried a carbapenemase gene, most frequently blaNDM (17; 63.0% of carbapenem-resistant isolates) and blaOXA-48 (11; 40.7%), with co-carriage in 6. blaOXA-23 was detected in 16 of 21 Acinetobacter baumannii (76.2%). Agreement between phenotype and genotype was almost perfect for all three comparisons: κ = 0.939 (95% CI 0.822–1.000) for cefoxitin versus mecA, κ = 0.903 (0.810–0.996) for the combined-disc test versus ESBL genes, and κ = 0.921 (0.832–1.000) for meropenem non-susceptibility versus carbapenemase genes. Emergency surgery (OR 3.09), preoperative stay beyond 48 hours (OR 3.06) and prior antimicrobial exposure (OR 3.71) were significantly associated with MDR SSI.

Conclusions: Post-surgical wound infections at this centre were dominated by multidrug-resistant Gram-negative bacilli carrying blaCTX-M and blaNDM determinants. Routine phenotypic methods showed almost perfect agreement with PCR and remain a dependable operational basis for therapy, but they missed a small number of gene-carrying isolates — including an oxacillin-susceptible mecA-positive S. aureus and two carbapenemase carriers that tested meropenem-susceptible — that are of consequence for infection control. Targeted molecular confirmation, applied selectively to discordant and epidemiologically important isolates, is a rational adjunct to phenotypic testing in Indian tertiary care laboratories.

KEYWORDS: Surgical site infection; multidrug resistance; extended-spectrum β-lactamase; carbapenemase; blaNDM; blaCTX-M; mecA; genotype–phenotype correlation; antimicrobial stewardship; India

1. INTRODUCTION

Surgical site infection (SSI) remains one of the most frequent and most preventable healthcare-associated infections worldwide. The World Health Organization's global guidelines for the prevention of surgical site infection identify SSI as the commonest healthcare-associated infection in low- and middle-income countries, and set out 29 evidence-based recommendations for its prevention [1]. A systematic review and meta-analysis of 488,594 general surgical patients placed the pooled worldwide incidence of SSI at 11% [2]. Indian data are broadly consistent but heterogeneous: a 24-month surveillance study of 25,809 surgeries in a Kolkata tertiary care centre reported an SSI rate of 6.3%, with 1,928 isolates recovered from 1,628 infections [3]. Beyond morbidity, the economic consequences are substantial; in a study of 2,024 caesarean sections in an Indian teaching hospital, the total cost of illness attributable to incisional SSI was almost three times that of matched uninfected controls [4].

What has changed over the past decade is not so much the frequency of SSI as the resistance profile of the organisms causing it. The Global Burden of Disease study estimated 4.71 million deaths associated with, and 1.14 million attributable to, bacterial antimicrobial resistance (AMR) in 2021, with a projected 8.22 million associated deaths by 2050 [5]. India carries a disproportionate share of this burden. The 2024 annual report of the Indian Council of Medical Research Antimicrobial Resistance Surveillance Network (ICMR-AMRSN) recorded methicillin resistance in 50.0% of 8,515 Staphylococcus aureus isolates, meropenem resistance in 64.9% of Klebsiella pneumoniae and 91.0% of Acinetobacter baumannii, and imipenem resistance in 42.4% of Escherichia coli [6]. The 2024 WHO Bacterial Priority Pathogens List places carbapenem-resistant Enterobacterales, third-generation cephalosporin-resistant Enterobacterales and carbapenem-resistant A. baumannii in the critical tier, with carbapenem-resistant K. pneumoniae scoring highest of the 24 pathogens assessed [7]. That these organisms are not merely of surveillance interest was shown by a retrospective analysis of 4,437 Indian patients, in which multidrug resistance carried an adjusted odds ratio for mortality of 1.57 and extensive drug resistance an odds ratio of 2.65 [8].

Resistance in these organisms is largely mediated by acquired, horizontally transferable determinants. In staphylococci, methicillin resistance is conferred by mecA, encoding the low-affinity penicillin-binding protein PBP2a. In Enterobacterales, extended-spectrum β-lactamase (ESBL) production is dominated in India by the blaCTX-M family, with blaTEM and blaSHV frequently co-carried, while carbapenem resistance is driven principally by the metallo-β-lactamase blaNDM and the class D carbapenemase blaOXA-48. In A. baumannii, acquired blaOXA-23 is the usual determinant of carbapenem resistance, whereas the intrinsic chromosomal blaOXA-51 serves as a species marker rather than an acquired resistance gene. Indian series bear this out: blaNDM was detected in 63.3% and blaOXA-48 in 61.2% of characterised carbapenem-resistant Enterobacterales in a Bihar tertiary centre [9]; blaTEM (49.4%), blaCTX-M group 1 (31.9%) and blaSHV (11.9%) predominated among 269 ESBL producers in a North Indian series [10]; and blaOXA-51 (97.7%) and blaOXA-23 (91.7%) were near-universal among 133 clinical A. baumannii isolates, of which 79% were extensively drug-resistant [11]. A pooled analysis of 98 studies published between 2015 and 2020 placed the prevalence of methicillin-resistant S. aureus (MRSA) in India at 37%, with a western-zone estimate of 33% [12].

For the working laboratory, this raises a practical question. Molecular characterisation defines the epidemiology of resistance and is indispensable for outbreak investigation and for tracking the spread of transferable determinants, but it is not available in most Indian district and medical-college laboratories, and it does not by itself yield a susceptibility report. Therapy continues to rest on phenotypic testing. The clinically relevant question is therefore not whether PCR is more sensitive in the abstract, but how closely routine phenotypic methods — the cefoxitin disc, the combined-disc ESBL confirmatory test, the modified carbapenem inactivation method — track the underlying genotype in the specific organisms causing post-surgical wound infection, and where they fail. Studies addressing this correlation directly remain relatively few in the Indian literature, and those that exist have largely examined urinary rather than surgical isolates [13].

Dadra and Nagar Haveli and Daman and Diu is a Union Territory whose tertiary surgical services have expanded rapidly, and for which published AMR data are sparse. We therefore undertook a prospective study, jointly conducted by the Departments of Microbiology and Surgery, with three objectives: to define the bacteriological profile and antimicrobial resistance pattern of pathogens recovered from post-surgical wound infections at our institute; to determine the prevalence of eleven clinically important β-lactam resistance determinants by PCR; and to quantify, using Cohen's κ, the agreement between phenotypic and genotypic detection of methicillin resistance, ESBL production and carbapenemase production. A secondary objective was to identify perioperative factors associated with multidrug-resistant SSI, to inform local prophylaxis and stewardship policy.

2. MATERIALS AND METHODS

2.1 Study design, setting and ethical approval

This was a prospective, cross-sectional, observational study conducted jointly by the Department of Microbiology and the Department of Surgery at NAMO Medical Education and Research Institute (NAMOMERI), Silvassa, a tertiary care teaching hospital serving the Union Territory of Dadra and Nagar Haveli and Daman and Diu and adjoining districts of Gujarat and Maharashtra. Patients were enrolled over twelve months, from July 2025 to June 2026, with post-discharge surveillance continuing to 31 July 2026 so that every enrolled patient completed a full 30-day postoperative follow-up window.

The protocol was reviewed and approved by the Institutional Ethics Committee of NAMOMERI. Written informed consent was obtained from every participant, or from a legally authorised representative where the patient was unable to consent. The study was conducted in accordance with the Declaration of Helsinki and the ICMR National Ethical Guidelines for Biomedical and Health Research Involving Human Participants. Patient identifiers were replaced by study codes at the point of data entry, and the linking key was held separately by the principal investigator.

Inclusion criteria. All patients aged 18 years or above undergoing an elective or emergency surgical procedure under the departments of General Surgery, Orthopaedics, Obstetrics and Gynaecology, Urology, Otorhinolaryngology, Ophthalmology or Plastic Surgery during the enrolment period, who consented to participate and who were available for 30-day postoperative follow-up.

Exclusion criteria. Patients with clinical or microbiological evidence of infection at the operative site before surgery; patients undergoing a procedure on a site already colonised or infected as part of the presenting illness where the infection could not be distinguished from a postoperative event; patients who died within 48 hours of surgery from causes unrelated to infection; and patients lost to follow-up before day 30. Repeat isolates of the same species with an identical antibiogram from the same patient were excluded so that only non-duplicate isolates entered the analysis.

2.2 Case definition and classification of surgical site infection

Surgical site infection was defined and classified according to the Centers for Disease Control and Prevention / National Healthcare Safety Network (CDC/NHSN) Patient Safety Component Manual criteria [14]. Infections were classified as superficial incisional (involving only skin and subcutaneous tissue), deep incisional (involving fascial and muscle layers), or organ/space (involving any part of the body deeper than the fascial and muscle layers that was opened or manipulated during the procedure). Because the operative case mix at this institute comprises procedures within NHSN's 30-day surveillance categories, a uniform 30-day post-procedure surveillance window was applied, with day 1 taken as the date of the procedure. The date of event was recorded as the date on which the first element used to satisfy the SSI criterion was first present.

Wounds were classified intraoperatively as clean, clean-contaminated, contaminated or dirty/infected by the operating surgeon. ASA physical status was assigned by the attending anaesthetist. Surveillance was performed by daily ward rounds during admission and by structured telephone and outpatient follow-up on days 7, 15 and 30 after discharge. Case ascertainment was performed independently by a surgical registrar and a microbiologist; discordant assessments were resolved by consensus with a third assessor.

2.3 Specimen collection and processing

Where SSI was suspected, the wound was cleansed with sterile normal saline and specimens were collected before any dressing change and, wherever clinically possible, before the initiation or escalation of antimicrobial therapy. Deep tissue or aspirated pus was preferred to superficial swabs; where a swab was unavoidable, two swabs were collected using the Levine technique. Specimens were transported to the laboratory within 30 minutes.

Specimens were inoculated onto 5% sheep blood agar, MacConkey agar and chocolate agar, and incubated aerobically at 37 °C for 18–24 hours, with chocolate agar incubated in 5% CO_2. Plates showing no growth were re-incubated for a further 24 hours before being reported as sterile. Gram staining was performed on all specimens. Routine anaerobic culture was not performed.

2.4 Identification and antimicrobial susceptibility testing

Isolates were identified to species level by colony morphology, Gram stain and conventional biochemical reactions, with confirmation on the VITEK 2 Compact automated system (bioMérieux, Marcy-l'Étoile, France) using GN and GP identification cards. Antimicrobial susceptibility was determined by automated broth microdilution on the VITEK 2 (AST-N and AST-P cards) and by Kirby–Bauer disc diffusion on Mueller–Hinton agar for agents not present on the cards. Results were interpreted using the breakpoints of the Clinical and Laboratory Standards Institute, CLSI supplement M100, 36th edition [15], which was the edition in force for the majority of the data-collection period. Colistin susceptibility was determined exclusively by broth microdilution, in accordance with the joint CLSI–EUCAST position that disc diffusion and gradient diffusion are unreliable for polymyxins.

Quality control was performed on each new lot of media and discs and at least weekly thereafter using Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853 and Klebsiella pneumoniae ATCC 700603.

Multidrug resistance (MDR), extensive drug resistance (XDR) and pandrug resistance (PDR) were assigned using the interim standard definitions of Magiorakos and colleagues [16]: MDR as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR as non-susceptibility to at least one agent in all but two or fewer categories, and PDR as non-susceptibility to all agents in all categories. Non-susceptibility included both intermediate and resistant results, and categories rather than individual agents were counted, using the organism-specific category lists given in that paper.

2.5 Phenotypic detection of resistance mechanisms

Methicillin resistance. Staphylococci were screened with a 30 µg cefoxitin disc on Mueller–Hinton agar incubated at 35 ± 2 °C for 16–18 hours, with zone diameters interpreted per CLSI M100 (S. aureus: ≤ 21 mm resistant).

ESBL production. Enterobacterales resistant to ceftriaxone, cefotaxime or ceftazidime were confirmed by the CLSI combined-disc method, comparing the inhibition zone of ceftazidime (30 µg) and cefotaxime (30 µg) alone with that of each drug combined with clavulanic acid (10 µg). An increase of ≥ 5 mm in the zone diameter of either combination over the corresponding agent alone was taken as confirmation of ESBL production.

Carbapenemase production. Isolates non-susceptible to meropenem or imipenem were tested by the modified carbapenem inactivation method (mCIM), with the EDTA-modified carbapenem inactivation method (eCIM) performed in parallel to distinguish metallo-β-lactamase production from serine carbapenemase production, both performed and interpreted as specified in CLSI M100 [15].

2.6 DNA extraction and molecular characterisation

Genomic DNA was extracted from overnight nutrient broth cultures using a commercial spin-column kit (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's protocol, with an additional lysostaphin pre-treatment step for staphylococci. DNA purity and concentration were assessed spectrophotometrically, and extracts with an A260/A280 ratio between 1.8 and 2.0 were retained. Extracts were stored at −20 °C until amplification.

All 187 isolates were subjected to PCR for the resistance determinants appropriate to their species, rather than only those isolates that screened positive phenotypically. This design decision is central to the concordance analysis: testing only phenotypically resistant isolates would make it impossible to detect gene-positive, phenotype-negative isolates and would bias the estimate of agreement.

Amplification was performed for mecA in staphylococci [17]; for blaCTX-M (groups 1 and 9), blaTEM and blaSHV in Enterobacterales using the multiplex scheme of Dallenne and colleagues [18]; for blaNDM, blaOXA-48, blaKPC, blaVIM and blaIMP using the single-reaction multiplex of Poirel and colleagues [19]; and for blaOXA-23 and blaOXA-51 in Acinetobacter baumannii using the scheme of Woodford and colleagues [20]. Primer sequences, amplicon sizes and annealing temperatures are given in Table 1. The Dallenne and Poirel schemes were not combined in a single reaction, since their amplicon sizes and annealing optima differ; a comparable application of the Dallenne panel to Indian isolates has been described [22], and mecA detection alongside nuc-based species confirmation has been applied to Indian MRSA isolates [21].

Reactions were performed in 25 µL volumes containing 12.5 µL of 2× PCR master mix, 0.4 µM of each primer and 2 µL of template DNA. Cycling for the carbapenemase multiplex followed Poirel and colleagues: 94 °C for 10 minutes, then 36 cycles of 94 °C for 30 seconds, 52 °C for 40 seconds and 72 °C for 50 seconds, with a final extension of 72 °C for 5 minutes [19]. The ESBL multiplex used an annealing temperature of 60 °C and the mecA singleplex 53 °C. Each run included a positive control of a previously characterised, sequence-confirmed isolate and a no-template negative control. Amplicons were resolved by electrophoresis on 1.5% agarose gel stained with ethidium bromide against a 100 bp ladder and visualised under ultraviolet transillumination. Representative amplicons of each gene target (approximately 10% of positives) were purified and subjected to Sanger sequencing for confirmation; sequences were compared with GenBank entries using BLASTn.

blaOXA-51 was interpreted as an intrinsic chromosomal species marker confirming the identity of A. baumannii, and not as an acquired carbapenem resistance determinant.

2.7 Statistical analysis

Data were entered into a structured spreadsheet and analysed in Python 3.11 (SciPy 1.17). Categorical variables are presented as frequencies and percentages, and continuous variables as mean ± standard deviation. Proportions are reported with 95% confidence intervals calculated by the Wilson score method.

Agreement between phenotypic and genotypic detection was quantified by Cohen's κ with 95% confidence intervals derived from the asymptotic standard error, and interpreted using the benchmarks of Landis and Koch [23], in which values above 0.80 denote almost perfect agreement. Taking PCR as the reference standard, the sensitivity, specificity, positive predictive value and negative predictive value of each phenotypic method were calculated, and the association within each 2 × 2 table was tested by Fisher's exact test. Because κ is unstable when the marginal distribution is highly skewed, κ was not computed for the mCIM comparison, which was restricted to the 27 meropenem-non-susceptible isolates; sensitivity and specificity are reported for that comparison instead.

Associations between perioperative variables and multidrug-resistant SSI were tested by Pearson's χ^2 test without continuity correction, with odds ratios and 95% confidence intervals calculated by the Woolf logit method; the smallest expected cell frequency across all contingency tables was 11.6, so the asymptotic test was valid throughout. Length of hospital stay was compared by Welch's unequal-variance t test and mortality by Fisher's exact test. A two-sided p value below 0.05 was taken as statistically significant. The analysis of perioperative variables was univariate; no multivariable model was fitted, and the associations reported should be read as unadjusted.

Table 1. Oligonucleotide primers used for the detection of antimicrobial resistance determinants.

Target	Primer sequences (5'→3'), forward / reverse	Amplicon (bp)	Annealing (°C)	Source
mecA	TCCAGATTACAACTTCACCAGG / CCACTTCATATCTTGTAACG	162	53	Oliveira & de Lencastre [17]

Target	Primer sequences (5'→3'), forward / reverse	Amplicon (bp)	Annealing (°C)	Source
blaCTX-M gp 1	TTAGGAARTGTGCCGCTGYA / CGATATCGTTGGTGGTRCCAT	688	60	Dallenne et al. [18]
blaCTX-M gp 9	TCAAGCCTGCCGATCTGGT / TGATTCTCGCCGCTGAAG	561	60	Dallenne et al. [18]
blaTEM	CATTTCCGTGTCGCCCTTATTC / CGTTCATCCATAGTTGCCTGAC	800	60	Dallenne et al. [18]
blaSHV	AGCCGCTTGAGCAAATTAAAC / ATCCCGCAGATAAATCACCAC	713	60	Dallenne et al. [18]
blaNDM	GGTTTGGCGATCTGGTTTTC / CGGAATGGCTCATCACGATC	621	52	Poirel et al. [19]
blaOXA-48	GCGTGGTTAAGGATGAACAC / CATCAAGTTCAACCCAACCG	438	52	Poirel et al. [19]
blaKPC	CGTCTAGTTCTGCTGTCTTG / CTTGTCATCCTTGTTAGGCG	798	52	Poirel et al. [19]
blaVIM	GATGGTGTTTGGTCGCATA / CGAATGCGCAGCACCAG	390	52	Poirel et al. [19]
blaIMP	GGAATAGAGTGGCTTAAYTCTC / GGTTTAAYAAAACAACCACC	232	52	Poirel et al. [19]
blaOXA-23	GATCGGATTGGAGAACCAGA / ATTTCTGACCGCATTTCCAT	501	52	Woodford et al. [20]
blaOXA-51	TAATGCTTTGATCGGCCTTG / TGGATTGCACTTCATCTTGG	353	52	Woodford et al. [20]

blaOXA-51 is intrinsic and chromosomal in Acinetobacter baumannii and was used as a species-confirmation marker, not as an acquired resistance determinant. Primer sequences are reproduced from the cited publications. Degenerate positions follow IUPAC convention (R = A/G; Y = C/T).

3. RESULTS

3.1 Burden of surgical site infection and patient characteristics

During the twelve-month enrolment period, 2,184 surgical procedures were performed and prospectively monitored. Surgical site infection was clinically suspected in 231 patients, of whom 178 satisfied the CDC/NHSN case definition, giving an overall SSI rate of 8.15% (95% CI 7.05–9.37). Of these 178 infections, 108 (60.7%) were superficial incisional, 51 (28.7%) deep incisional and 19 (10.7%) organ/space. The study profile is shown in Figure 1.

Cultures were positive in 156 of the 178 cases (87.6%); the remaining 22 yielded no growth, in most instances following antimicrobial therapy started before specimen collection. Of the 156 culture-positive infections, 127 (81.4%) were monomicrobial and 29 (18.6%) polymicrobial, yielding 187 non-duplicate isolates at a mean of 1.20 isolates per culture-positive case.

The demographic and perioperative characteristics of the 156 culture-positive patients are summarised in Table 2. Ninety-two (59.0%) were male and 64 (41.0%) female, with a mean age of 47.3 ± 15.8 years; 61 patients (39.1%) were aged 41–60 years. General surgery accounted for 71 cases (45.5%), obstetrics and gynaecology for 34 (21.8%) and orthopaedics for 28 (17.9%). Ninety patients (57.7%) had undergone emergency rather than elective procedures, and 75 (48.1%) had a contaminated or dirty wound class.

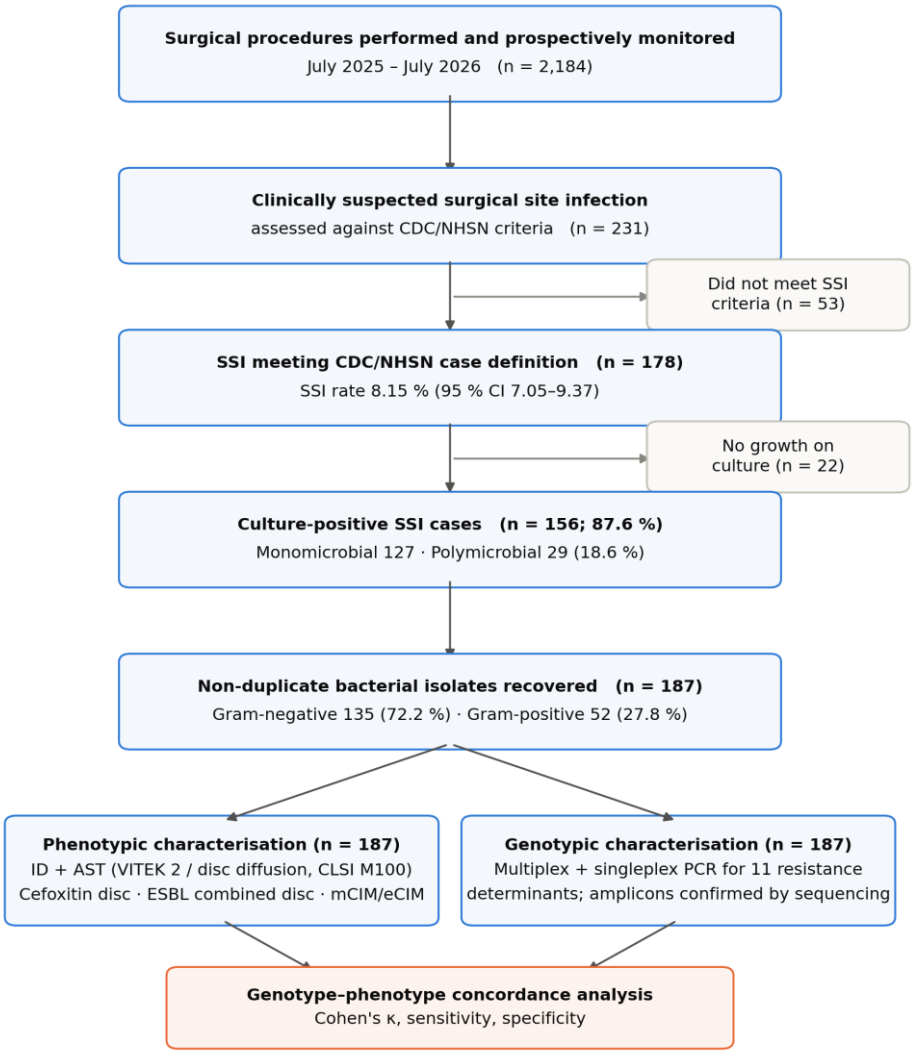

Figure 1. Study profile. Flow of patients from surgical procedures monitored through case ascertainment, culture, and phenotypic and genotypic characterisation, to the concordance analysis.

Table 2. Demographic and perioperative characteristics of patients with culture-positive surgical site infection (n = 156).

Characteristic	n	%
Sex — male	92	59.0
Sex — female	64	41.0
Age ≤ 20 years	9	5.8
Age 21–40 years	48	30.8
Age 41–60 years	61	39.1
Age > 60 years	38	24.4
Surgical specialty — General surgery	71	45.5
Surgical specialty — Obstetrics and gynaecology	34	21.8
Surgical specialty — Orthopaedics	28	17.9
Surgical specialty — Urology	13	8.3
Surgical specialty — Other	10	6.4
Emergency (non-elective) procedure	90	57.7
Contaminated or dirty wound class	75	48.1
Operative duration > 2 hours	79	50.6
Preoperative stay > 48 hours	95	60.9
Prior antimicrobial exposure	80	51.3

Characteristic	n	%
ASA physical status ≥ III	53	34.0
Diabetes mellitus	61	39.1
Re-exploration or re-operation	33	21.2

Mean age 47.3 ± 15.8 years. Percentages are of the 156 culture-positive cases. Prior antimicrobial exposure was defined as ≥ 7 days of systemic antimicrobial therapy within the 90 days preceding surgery.

3.2 Bacteriological profile

Gram-negative bacilli accounted for 135 of the 187 isolates (72.2%) and Gram-positive cocci for 52 (27.8%). Klebsiella pneumoniae was the commonest isolate overall (38; 20.3%), followed by Escherichia coli (34; 18.2%), Staphylococcus aureus (33; 17.6%), Pseudomonas aeruginosa (26; 13.9%) and Acinetobacter baumannii (21; 11.2%). The full distribution of isolates is set out in Table 3, and the rank order and Gram split are shown graphically in Figure 2. The 88 Enterobacterales isolates — K. pneumoniae, E. coli, Proteus mirabilis and Enterobacter cloacae — formed the denominator for all subsequent ESBL and carbapenemase analyses.

Table 3. Distribution of bacterial isolates recovered from surgical site infections (n = 187).

Organism	Gram reaction	n	% of isolates
Klebsiella pneumoniae	Gram-negative bacilli	38	20.32
Escherichia coli	Gram-negative bacilli	34	18.18
Staphylococcus aureus	Gram-positive cocci	33	17.65
Pseudomonas aeruginosa	Gram-negative bacilli	26	13.90
Acinetobacter baumannii	Gram-negative bacilli	21	11.23
Coagulase-negative staphylococci	Gram-positive cocci	11	5.88
Proteus mirabilis	Gram-negative bacilli	9	4.81
Enterococcus faecalis	Gram-positive cocci	8	4.28
Enterobacter cloacae	Gram-negative bacilli	7	3.74
Total		**187**	**100.00**

Isolates were recovered from 156 culture-positive infections, of which 29 (18.6%) were polymicrobial.

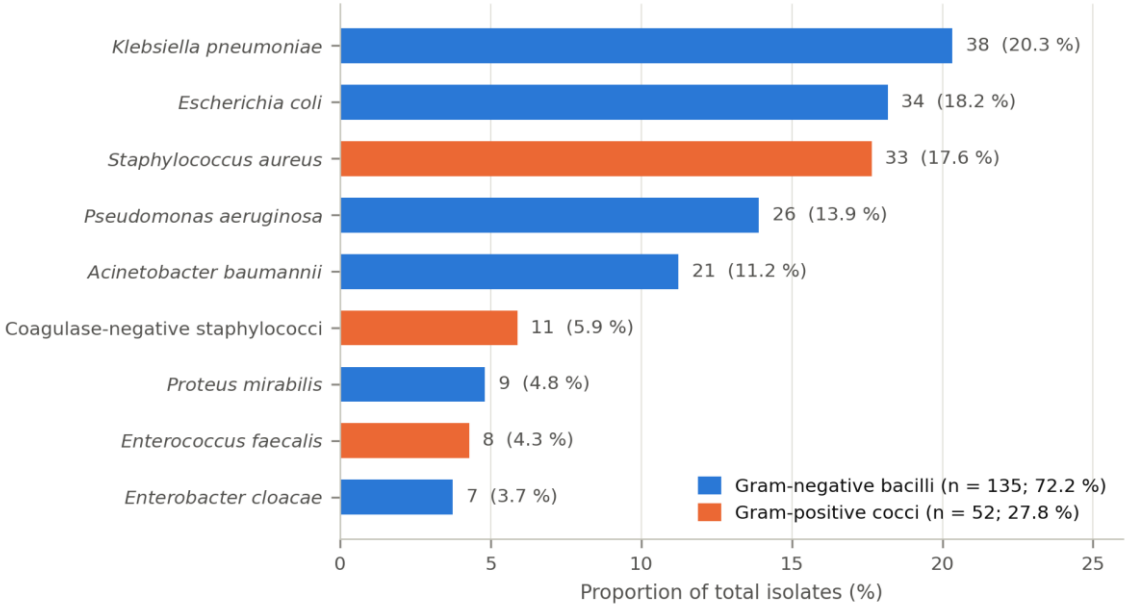

Figure 2. Distribution of the 187 non-duplicate bacterial isolates recovered from culture-positive surgical site infections, coloured by Gram reaction. Values beside each bar give the isolate count and the percentage of all isolates.

3.3 Antimicrobial susceptibility

Susceptibility profiles are given in Table 4 for Gram-negative bacilli and Table 5 for Gram-positive cocci, and the two profiles are summarised together as panels A and B of Figure 3, in which cell shading is proportional to the percentage of isolates non-susceptible. Among Gram-negative isolates, susceptibility to third- and fourth-generation cephalosporins was uniformly poor: 21.1% of K. pneumoniae and 32.4% of E. coli were susceptible

to ceftriaxone. Carbapenem susceptibility was better preserved in E. coli (76.5% to both meropenem and imipenem) than in K. pneumoniae (63.2% and 65.8%). A. baumannii was the most resistant organism encountered, with only 19.0% susceptibility to carbapenems, cefepime and piperacillin–tazobactam. Colistin retained near-complete activity against all Gram-negative isolates tested (95.2–100%), and tigecycline retained good activity against Enterobacterales (85.7–94.1%) and moderate activity against A. baumannii (76.2%).

Among Gram-positive isolates (Table 5; Figure 3B), no resistance to linezolid was detected in any organism, and vancomycin and teicoplanin retained complete activity against staphylococci. Penicillin susceptibility was minimal (12.1% of S. aureus, 9.1% of coagulase-negative staphylococci). A single Enterococcus faecalis isolate (12.5%) was vancomycin-resistant.

Table 4. Antimicrobial susceptibility of Gram-negative bacilli isolated from surgical site infections (percentage susceptible).

Organism (n)	AK	GEN	CIP	CRO/CAZ	FEP	TZP	IPM	MEM	TGC	CST	SXT
Klebsiella pneumoniae (38)	47.4	39.5	26.3	21.1	28.9	34.2	65.8	63.2	89.5	97.4	31.6
Escherichia coli (34)	61.8	47.1	23.5	32.4	38.2	44.1	76.5	76.5	94.1	100.0	35.3
Pseudomonas aeruginosa (26)	61.5	50.0	46.2	53.8	57.7	61.5	57.7	57.7	—	96.2	—
Acinetobacter baumannii (21)	28.6	23.8	19.0	14.3	19.0	19.0	19.0	19.0	76.2	95.2	23.8
Proteus mirabilis (9)	66.7	55.6	44.4	44.4	55.6	66.7	77.8	77.8	—	—	44.4
Enterobacter cloacae (7)	57.1	42.9	42.9	28.6	42.9	42.9	57.1	57.1	85.7	100.0	42.9

AK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; CRO/CAZ, ceftriaxone (Enterobacterales) or ceftazidime (non-fermenters); FEP, cefepime; TZP, piperacillin–tazobactam; IPM, imipenem; MEM, meropenem; TGC, tigecycline; CST, colistin; SXT, trimethoprim–sulfamethoxazole. Em dash denotes an agent not tested or without an applicable breakpoint. Colistin was tested by broth microdilution only. Interpretation per CLSI M100, 36th edition.

Table 5. Antimicrobial susceptibility of Gram-positive cocci isolated from surgical site infections (percentage susceptible).

Organism (n)	P	FOX	E	DA	CIP	GEN	SXT	DO	LZD	VA	TEC
Staphylococcus aureus (33)	12.1	54.5	39.4	54.5	45.5	60.6	63.6	78.8	100.0	100.0	100.0
Coagulase-negative staphylococci (11)	9.1	27.3	36.4	45.5	36.4	54.5	54.5	72.7	100.0	100.0	100.0
Enterococcus faecalis (8)	75.0	—	—	—	50.0	62.5	—	75.0	100.0	87.5	87.5

P, penicillin (ampicillin for E. faecalis); FOX, cefoxitin; E, erythromycin; DA, clindamycin; CIP, ciprofloxacin; GEN, gentamicin (high-level gentamicin for E. faecalis); SXT, trimethoprim–sulfamethoxazole; DO, doxycycline; LZD, linezolid; VA, vancomycin; TEC, teicoplanin. Em dash denotes an agent not tested or without an applicable breakpoint.

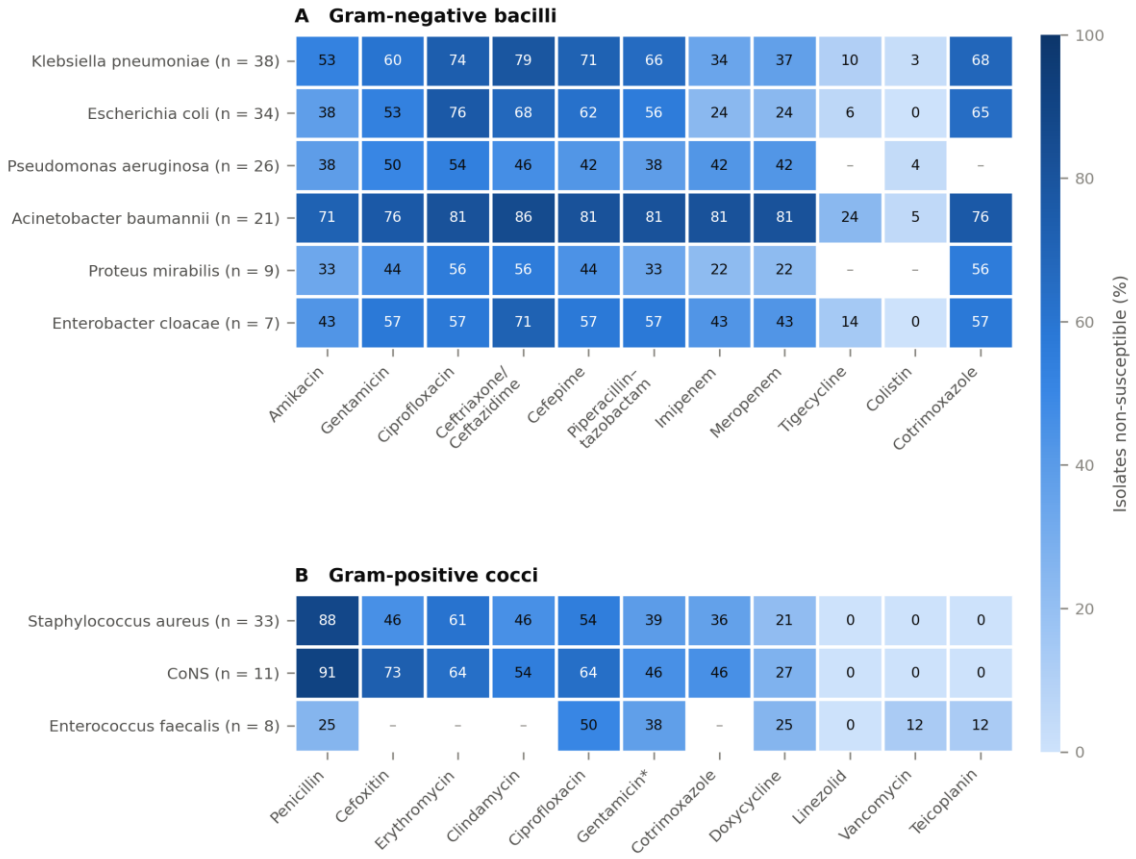

Figure 3. Antimicrobial non-susceptibility of (A) Gram-negative bacilli and (B) Gram-positive cocci from surgical site infections. Cell values are the percentage of isolates non-susceptible (intermediate plus resistant); darker cells indicate greater resistance. Blank cells denote agents not tested or without an applicable breakpoint.

3.4 Multidrug resistance

One hundred and seventeen of the 187 isolates (62.6%) met the criteria for multidrug resistance. Species-level rates are given in Table 6. A. baumannii showed the highest proportion (18/21; 85.7%), of which 9 isolates (42.9% of all A. baumannii) met the criteria for extensive drug resistance. K. pneumoniae followed at 73.7% (28/38), then coagulase-negative staphylococci at 72.7% (8/11), E. cloacae at 71.4% (5/7) and E. coli at 67.6% (23/34). No pandrug-resistant isolate was identified.

The phenotypic resistance mechanisms underlying these classifications are also given in Table 6. Phenotypic screening identified 15 methicillin-resistant S. aureus isolates (45.5% of S. aureus) and 8 methicillin-resistant coagulase-negative staphylococci (72.7%). Among the 88 Enterobacterales, 54 (61.4%) were confirmed ESBL producers by the combined-disc method — 25 of 38 K. pneumoniae (65.8%), 21 of 34 E. coli (61.8%), 4 of 9 P. mirabilis (44.4%) and 4 of 7 E. cloacae (57.1%) — and 27 (30.7%) were non-susceptible to meropenem. Of these 27, mCIM was positive in 25, and eCIM indicated metallo-β-lactamase production in 19.

Table 6. Phenotypic resistance mechanisms and multidrug-resistance classification by species, n (%).

Organism	n	ESBL / methicillin resistance	Carbapenem non-susceptible	MDR	XDR
Klebsiella pneumoniae	38	25 (65.8)	14 (36.8)	28 (73.7)	0
Escherichia coli	34	21 (61.8)	8 (23.5)	23 (67.6)	0
Pseudomonas aeruginosa	26	—	11 (42.3)	12 (46.2)	0
Acinetobacter baumannii	21	—	17 (81.0)	18 (85.7)	9 (42.9)
Proteus mirabilis	9	4 (44.4)	2 (22.2)	4 (44.4)	0
Enterobacter cloacae	7	4 (57.1)	3 (42.9)	5 (71.4)	0
Staphylococcus aureus	33	15 (45.5)[a]	—	16 (48.5)	0
Coagulase-negative staphylococci	11	8 (72.7)[a]	—	8 (72.7)	0
Enterococcus faecalis	8	1 (12.5)[b]	—	3 (37.5)	0

Organism	n	ESBL / methicillin resistance	Carbapenem non-susceptible	MDR	XDR
Total	**187**			**117 (62.6)**	**9 (4.8)**

[a] Methicillin resistance by cefoxitin disc diffusion. [b] Vancomycin resistance. MDR and XDR assigned per Magiorakos et al. [16]. No pandrug-resistant isolate was identified. Em dash denotes a mechanism not applicable to that organism.

3.5 Molecular characterisation of resistance determinants

The full distribution of resistance determinants is given in Table 7; the ESBL determinants are shown in Figure 4A and the carbapenemase determinants in Figure 4B. mecA was detected in 16 of 33 S. aureus (48.5%) and in 8 of 11 coagulase-negative staphylococci (72.7%).

Among the 88 Enterobacterales, 56 (63.6%) carried at least one ESBL determinant. blaCTX-M was the commonest, present in 47 isolates (53.4% of all Enterobacterales; 83.9% of gene-positive isolates), followed by blaTEM in 39 (44.3%) and blaSHV in 22 (25.0%). Co-carriage of two or more ESBL determinants was frequent. A carbapenemase determinant was detected in 28 Enterobacterales (31.8%). Among the 27 meropenem-non-susceptible isolates, blaNDM was present in 17 (63.0%) and blaOXA-48 in 11 (40.7%), with both genes co-carried by 6 isolates (22.2%); blaIMP was found in 2 (7.4%), and blaVIM and blaKPC in 1 each (3.7%). One meropenem-non-susceptible isolate carried none of the five targeted carbapenemase genes.

As shown in the non-fermenter series of Figure 4B, all 21 A. baumannii isolates carried blaOXA-51, consistent with its role as an intrinsic species marker. Acquired blaOXA-23 was present in 16 isolates (76.2%), accounting for 16 of the 17 carbapenem-resistant isolates (94.1%); blaNDM was co-carried by 8 (38.1%) and blaOXA-24/40 by 2 (9.5%). blaOXA-58 was not detected. Among the 26 P. aeruginosa isolates, 11 (42.3%) were carbapenem-resistant, and a metallo-β-lactamase gene was identified in 9 of these (81.8%): blaVIM in 5, blaNDM in 3 and blaIMP in 2.

Table 7. Antimicrobial resistance determinants detected by polymerase chain reaction.

Determinant	Denominator (n)	n	%	Comment
mecA	Staphylococci (44)	24	54.5	16 of 33 S. aureus; 8 of 11 CoNS
blaCTX-M	Enterobacterales (88)	47	53.4	Commonest ESBL determinant
blaTEM	Enterobacterales (88)	39	44.3	Frequently co-carried with blaCTX-M
blaSHV	Enterobacterales (88)	22	25.0	—
Any ESBL gene	Enterobacterales (88)	56	63.6	—
blaNDM	Carbapenem-R Enterobacterales (27)	17	63.0	Also in 8 A. baumannii, 3 P. aeruginosa
blaOXA-48	Carbapenem-R Enterobacterales (27)	11	40.7	Co-carried with blaNDM in 6
blaIMP	Carbapenem-R Enterobacterales (27)	2	7.4	Also in 2 P. aeruginosa
blaVIM	Carbapenem-R Enterobacterales (27)	1	3.7	Also in 5 P. aeruginosa
blaKPC	Carbapenem-R Enterobacterales (27)	1	3.7	—
blaOXA-51	A. baumannii (21)	21	100.0	Intrinsic species marker
blaOXA-23	A. baumannii (21)	16	76.2	16 of 17 carbapenem-resistant isolates
blaOXA-24/40	A. baumannii (21)	2	9.5	—
blaOXA-58	A. baumannii (21)	0	0.0	Not detected

Percentages are of the denominator stated in the second column. blaOXA-51 is intrinsic to A. baumannii and is reported for species confirmation only.

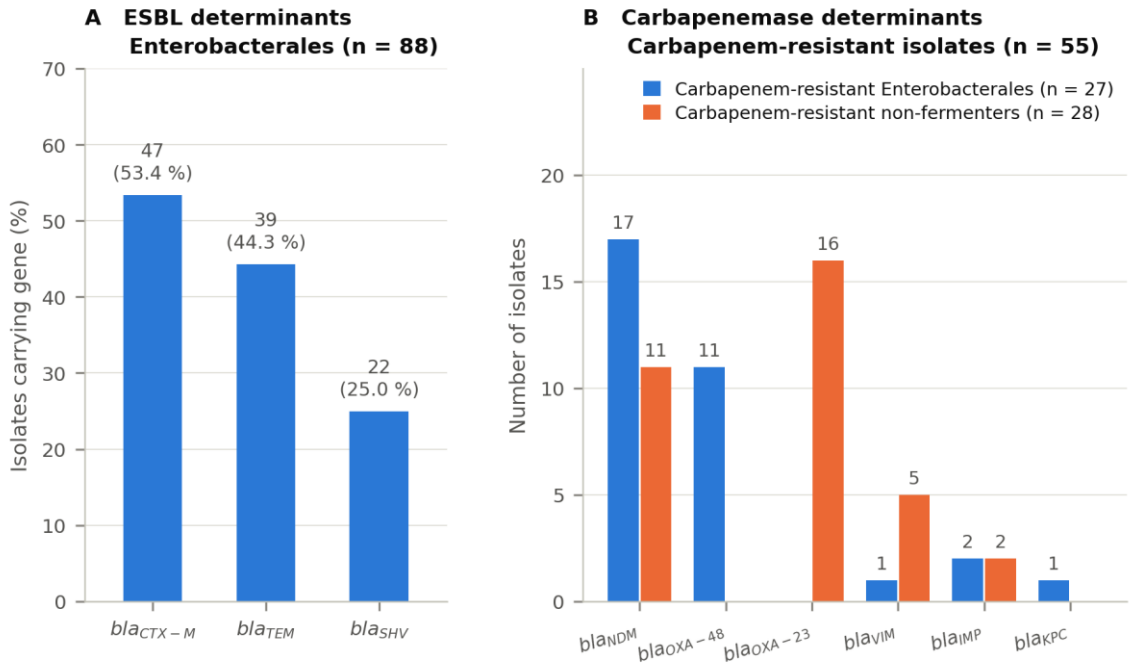

Figure 4. Resistance determinants detected by PCR. (A) ESBL determinants among all 88 Enterobacterales. (B) Carbapenemase determinants among carbapenem-resistant isolates, separated into Enterobacterales (n = 27) and non-fermentative Gram-negative bacilli (n = 28; 17 Acinetobacter baumannii and 11 Pseudomonas aeruginosa). Isolates carrying more than one determinant are counted in each bar.

3.6 Genotype–phenotype correlation

The full 2 × 2 cell counts and derived test characteristics for all three comparisons are given in Table 8. Figure 5A contrasts the proportion of isolates called resistant by each phenotypic method with the proportion carrying the corresponding determinant, and Figure 5B plots the three κ values with their confidence intervals against the Landis and Koch benchmark. Agreement was almost perfect for all three comparisons.

For methicillin resistance in S. aureus, the cefoxitin disc test and mecA PCR agreed in 32 of 33 isolates (97.0%), giving κ = 0.939 (95% CI 0.822–1.000). Taking PCR as the reference standard, the cefoxitin disc had a sensitivity of 93.8% and a specificity of 100.0%. The single discordant isolate was mecA-positive but cefoxitin-susceptible, consistent with an oxacillin-susceptible MRSA phenotype. No isolate was cefoxitin-resistant without mecA.

For ESBL production, the combined-disc test and ESBL gene PCR agreed in 84 of 88 Enterobacterales (95.5%), giving κ = 0.903 (95% CI 0.810–0.996), with a sensitivity of 94.6% and specificity of 96.9%. Three isolates carried an ESBL determinant without a confirmatory phenotype, and one isolate was phenotypically positive with no determinant detected on the panel used.

For carbapenemase production (Table 8, row 3), meropenem non-susceptibility and carbapenemase gene PCR agreed in 85 of 88 isolates (96.6%), giving κ = 0.921 (95% CI 0.832–1.000), with a sensitivity of 92.9% and specificity of 98.3%. Two meropenem-susceptible isolates carried blaOXA-48, and one meropenem-non-susceptible isolate carried no targeted carbapenemase gene. Within the 27 meropenem-non-susceptible isolates, mCIM identified 25 of the 26 gene-positive isolates (sensitivity 96.2%) with a specificity of 100%.

Table 8. Concordance between phenotypic and genotypic detection of resistance, with polymerase chain reaction as the reference standard.

Comparison	P+G+	P+G−	P−G+	P−G−	Agree (%)	Cohen's κ (95% CI)	Se (%)	Sp (%)	PPV (%)	NPV (%)
Cefoxitin disc vs mecA — S. aureus (n = 33)	15	0	1	17	97.0	0.939 (0.822–1.000)	93.8	100.0	100.0	94.4
Combined disc vs ESBL genes — Enterobacterales (n = 88)	53	1	3	31	95.5	0.903 (0.810–0.996)	94.6	96.9	98.2	91.2
Meropenem NS vs carbapenemase genes — Enterobacterales (n = 88)	26	1	2	59	96.6	0.921 (0.832–1.000)	92.9	98.3	96.3	96.7

P, phenotypic result; G, genotypic (PCR) result. Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value. All three associations were significant by Fisher's exact test ($p < 0.001$). κ

interpreted per Landis and Koch [23]. Within the 27 meropenem-non-susceptible isolates, mCIM had a sensitivity of 96.2% and a specificity of 100% against PCR; κ was not computed for that comparison because of the highly skewed marginal distribution.

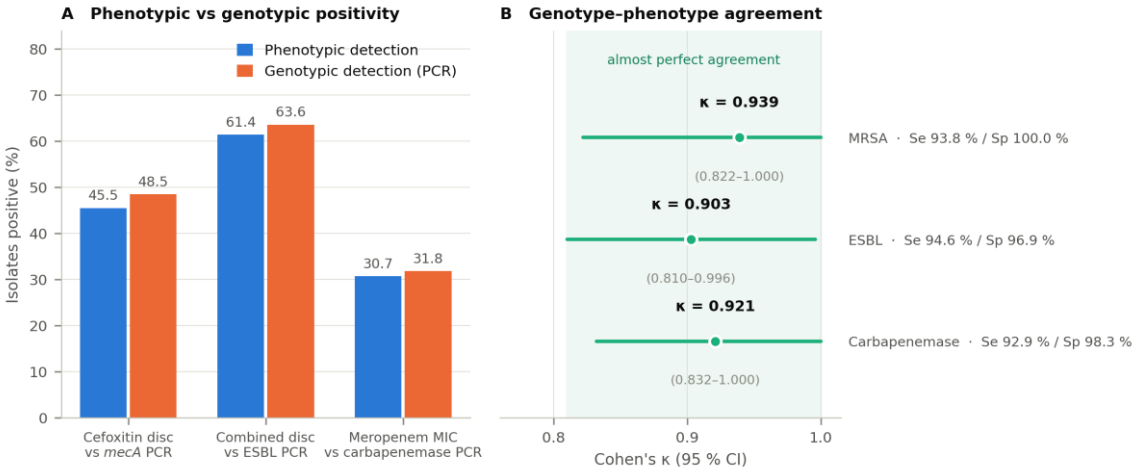

Figure 5. Genotype–phenotype concordance. (A) Proportion of isolates identified as resistant by the phenotypic method and by PCR for each of the three comparisons. (B) Cohen's κ with 95% confidence intervals; the shaded band marks the "almost perfect" range (κ > 0.80) of Landis and Koch. Se, sensitivity; Sp, specificity, both against PCR as reference standard.

3.7 Factors associated with multidrug-resistant surgical site infection

Of the 156 culture-positive patients, 101 (64.7%) had at least one multidrug-resistant isolate. The univariate associations for all eight perioperative variables examined are given in Table 9. Prior antimicrobial exposure showed the strongest association (62.4% versus 30.9%; OR 3.71, 95% CI 1.84–7.46; $\chi^2 = 14.113$, $p < 0.001$), followed by emergency surgery (OR 3.09; $p < 0.001$) and preoperative stay exceeding 48 hours (OR 3.06; p 0.001). Contaminated or dirty wound class (OR 2.66; p 0.005), operative duration beyond two hours (OR 2.46; p 0.008), re-exploration (OR 2.98; p 0.021), ASA physical status III or above (OR 2.45; p 0.018) and diabetes mellitus (OR 2.23; p 0.025) were also significantly associated. These associations are unadjusted; the variables are correlated with one another and no multivariable model was fitted.

Table 9. Perioperative factors associated with multidrug-resistant surgical site infection (univariate analysis, n = 156).

Factor	MDR SSI (n = 101), n (%)	Non-MDR SSI (n = 55), n (%)	χ^2	p	OR (95% CI)
Emergency (non-elective) surgery	68 (67.3)	22 (40.0)	10.894	< 0.001	3.09 (1.56–6.11)
Preoperative stay > 48 h	71 (70.3)	24 (43.6)	10.629	0.001	3.06 (1.54–6.05)
Prior antimicrobial exposure*	63 (62.4)	17 (30.9)	14.113	< 0.001	3.71 (1.84–7.46)
Operative duration > 2 h	59 (58.4)	20 (36.4)	6.928	0.008	2.46 (1.25–4.84)
Contaminated / dirty wound class	57 (56.4)	18 (32.7)	8.018	0.005	2.66 (1.34–5.29)
Diabetes mellitus	46 (45.5)	15 (27.3)	4.993	0.025	2.23 (1.10–4.54)
ASA physical status >= III	41 (40.6)	12 (21.8)	5.596	0.018	2.45 (1.15–5.20)
Re-exploration / re-operation	27 (26.7)	6 (10.9)	5.346	0.021	2.98 (1.15–7.75)

* Prior antimicrobial exposure defined as ≥ 7 days of systemic antimicrobial therapy within the 90 days preceding surgery. Pearson's χ^2 test, 1 degree of freedom, without continuity correction; smallest expected cell frequency 11.63. Odds ratios by the Woolf logit method. Associations are unadjusted; no multivariable model was fitted.

3.8 Clinical outcomes

Patients with multidrug-resistant SSI had a substantially longer postoperative hospital stay than those with non-MDR infection (18.4 ± 6.2 versus 11.7 ± 4.3 days; Welch $t = 7.914$, $p < 0.001$). All-cause in-hospital mortality was 7 of 101 (6.9%) in the MDR group and 1 of 55 (1.8%) in the non-MDR group; the difference was in the expected direction but did not reach statistical significance (OR 4.02; Fisher's exact $p = 0.262$), and the study was not powered for this outcome.

4. DISCUSSION

4.1 Principal findings

In this 12-month prospective study of 2,184 surgical procedures at a tertiary care institute in Silvassa, 8.15% of patients developed a surgical site infection meeting CDC/NHSN criteria. Gram-negative bacilli accounted for nearly three-quarters of the 187 isolates recovered, and 62.6% of isolates were multidrug-resistant. Molecular characterisation showed that this resistance was overwhelmingly enzymatic and transferable: blaCTX-M in 53.4% of Enterobacterales, blaNDM in 63.0% and blaOXA-48 in 40.7% of carbapenem-resistant Enterobacterales, and blaOXA-23 in 76.2% of A. baumannii. Against this genotypic background, routine phenotypic methods performed well (Table 8, Figure 5), with κ values between 0.903 and 0.939 — 'almost perfect' agreement on the Landis and Koch scale — for all three comparisons.

The central message of these data is therefore a reassuring one with an important qualification. For the purpose of guiding therapy, the phenotypic methods already in use in our laboratory identified the great majority of gene-carrying isolates, and a laboratory without molecular capability is not systematically misdirecting treatment. But the discordant isolates were not random noise. Each of the four discordances was of a recognised and clinically meaningful type, and each was of greater consequence for infection control than for the individual patient.

4.2 Bacteriological profile in context

The predominance of Gram-negative bacilli (72.2%; Table 3, Figure 2) closely matches the Kolkata series, in which Gram-negative organisms comprised 75.2% of 1,928 SSI isolates, with K. pneumoniae (26.3%) and E. coli (25.6%) leading [3]. Our leading isolates were the same two organisms in the same order (20.3% and 18.2%), and our S. aureus proportion (17.6%) sits between the Kolkata figure and the 21.6% reported from Western Gujarat, where K. pneumoniae (23.5%), S. aureus (21.6%) and E. coli (20.6%) were similarly ranked [24]. This consistency across three widely separated Indian centres suggests a stable national pattern of SSI aetiology dominated by Enterobacterales, and argues against the older teaching that S. aureus is the default SSI pathogen.

Our SSI rate of 8.15% falls between the Kolkata rate of 6.3% [3] and the pooled global estimate of 11% [2]. It is, however, an order of magnitude above the 0.23% reported from an Andhra Pradesh centre [25], and the discrepancy is instructive: that study counted only culture-confirmed infections against a denominator of all 21,952 surgeries performed, whereas we and the Kolkata investigators applied a clinical case definition prospectively. Comparisons of SSI rates between Indian centres are of limited value unless the case-ascertainment method and denominator are stated explicitly, and we would encourage authors to report both.

The polymicrobial rate of 18.6% and the mean of 1.20 isolates per culture-positive case are consistent with the general surgical and obstetric case mix at our institute, in which contaminated and dirty wounds — 48.1% of culture-positive cases — are well represented.

4.3 Phenotypic resistance and its molecular basis

MRSA accounted for 45.5% of S. aureus isolates. This exceeds the pooled Indian estimate of 37% and the western-zone estimate of 33% from a meta-analysis of 98 studies [12], but is closely comparable to the 43.9% reported among SSI isolates in Kolkata [3] and remains below the 50.0% recorded across all specimen types in the ICMR-AMRSN 2024 report [6]. Hospital-acquired surgical isolates would be expected to sit at the upper end of the community-inclusive pooled range, and our figure is consistent with that expectation rather than anomalous.

Among Enterobacterales, 61.4% were phenotypic ESBL producers. This is higher than the 40.3% reported among 2,312 K. pneumoniae isolates from Western Maharashtra [26] but well below the 79.1% found in healthcare-associated urinary isolates in North India [10] and the 88.3% reported among E. coli at another North Indian tertiary centre [27]. The gene distribution we observed — blaCTX-M in 53.4%, blaTEM in 44.3%, blaSHV in 25.0% of all Enterobacterales — places blaCTX-M first, in contrast to the North Indian series in which blaTEM (49.4%) exceeded blaCTX-M group 1 (31.9%) [10], but in agreement with that series (blaTEM 67.3%, blaCTX-M 63.3%) in showing extensive co-carriage [27]. The predominance of blaCTX-M is consistent with the global displacement of TEM- and SHV-derived ESBLs by the CTX-M family over the past two decades.

Carbapenem non-susceptibility in 30.7% of Enterobacterales corresponds almost exactly to the 32.97% reported among 3,421 Enterobacterales in Bihar [9], and our carbapenemase gene distribution (Table 7, Figure 4B) reproduces that study's findings with striking fidelity: blaNDM in 63.0% versus 63.3%, blaOXA-48 in 40.7% versus 61.2%, with blaIMP, blaVIM and blaKPC each below 8%. Co-carriage of blaNDM and blaOXA-48 in 6 of 27 carbapenem-resistant isolates (22.2%) is comparable to the 27.5% class A plus class B co-expression reported in that series. The therapeutic implication is direct: a metallo-β-lactamase such as NDM is not inhibited by avibactam, vaborbactam or relebactam, so the newer β-lactam–β-lactamase inhibitor combinations offer little against the dominant mechanism at our centre, and the aztreonam–ceftazidime-avibactam combination or colistin-based regimens remain the realistic options.

In A. baumannii, 81.0% of isolates were carbapenem-resistant and blaOXA-23 was present in 94.1% of those. This matches the 92.6% blaOXA-23 prevalence found across 339 Indian A. baumannii genomes [28] and the 91.7% reported in a separate Indian clinical isolate series [11]. We detected blaOXA-51 in all 21 isolates, as expected: it is intrinsic and chromosomal in A. baumannii and confirms species identity rather than indicating acquired resistance. We emphasise this because the contrary interpretation appears with some regularity in the literature, and reporting blaOXA-51 as a resistance determinant inflates apparent gene prevalence without adding information. Our carbapenem resistance rate is somewhat above the 66.4% meropenem resistance reported from a South Indian ICU series [29] and below the 91.0% in ICMR-AMRSN [6].

4.4 Genotype–phenotype concordance

All three phenotypic methods showed almost perfect agreement with PCR. The cefoxitin disc test achieved κ = 0.939 with 100% specificity, and the single false-negative was an oxacillin-susceptible mecA-positive S. aureus (OS-MRSA) — an isolate carrying mecA but expressing it insufficiently to produce cefoxitin resistance in vitro. OS-MRSA is a recognised phenomenon and a recognised diagnostic trap: such isolates may be reported as methicillin-susceptible, treated with a β-lactam, and select for full expression under therapy. Our finding of one such isolate among 33 (3.0%) is consistent with the low but non-zero rates described elsewhere, and supports the practice of confirming mecA in S. aureus isolated from deep or organ/space infections where β-lactam therapy is planned.

The combined-disc ESBL test achieved κ = 0.903, with three gene-positive isolates that tested phenotypically negative and one phenotypically positive isolate in which no ESBL gene was detected. The three false negatives are most plausibly explained by low-level expression or by masking through co-produced AmpC β-lactamase, which reduces the apparent clavulanate potentiation on which the combined-disc test depends. The single false positive is likely to reflect hyperproduction of a chromosomal or plasmid-mediated AmpC enzyme, or an ESBL variant outside the blaCTX-M, blaTEM and blaSHV families we targeted — a real limitation of any closed primer panel, discussed further below.

Meropenem non-susceptibility against carbapenemase PCR gave κ = 0.921. Two carbapenemase-gene-positive isolates were meropenem-susceptible; both carried blaOXA-48, whose weak carbapenemase activity is well described and which characteristically produces MICs at or just below the susceptibility breakpoint. These are precisely the isolates that phenotypic testing is expected to miss, and they matter: a patient may be treated successfully with a carbapenem while the isolate silently disseminates a transferable blaOXA-48 plasmid through the surgical ward. One meropenem-non-susceptible isolate carried no carbapenemase gene, and in that isolate resistance is likely to be mediated by porin loss combined with AmpC or ESBL hyperproduction rather than by carbapenemase production. Within the carbapenem-resistant subset, mCIM identified 25 of 26 gene-positive isolates (96.2%) with complete specificity, confirming its value as an accessible confirmatory assay where PCR is unavailable.

Taken together, these results support a selective rather than a universal molecular strategy. Routine phenotypic testing is adequate for therapeutic decision-making in the large majority of isolates. Molecular confirmation adds most where the consequence of a missed determinant is transmission rather than treatment failure: S. aureus from deep and organ/space infections, Enterobacterales with meropenem MICs in the intermediate or upper-susceptible range, and any isolate implicated in a suspected cluster. This is a defensible allocation of scarce molecular capacity in a resource-constrained laboratory.

4.5 Risk factors and stewardship implications

Prior antimicrobial exposure was the strongest correlate of MDR SSI (Table 9; OR 3.71; 95% CI 1.84–7.46), followed by emergency surgery (OR 3.09) and preoperative stay exceeding 48 hours (OR 3.06). None of these is novel, but their consistency is actionable at institutional level. Preoperative stay and prior antimicrobial exposure are both, in principle, modifiable. The association with emergency surgery is partly a proxy for contaminated and dirty wound class, which was itself significantly associated (OR 2.66), and partly for the reduced opportunity for optimisation in urgent cases.

These findings should be read alongside the evidence that antimicrobial stewardship in Indian hospitals remains underdeveloped below the tertiary tier. A mixed-methods needs assessment across eight primary and secondary hospitals in West Bengal found antibiotic prescription rates of 63.8% and 60.8% respectively, and identified that routine infection-control activity in lower-tier hospitals is largely delinked from AMR containment measures [30]. Our institute functions as a referral centre for such facilities, and a proportion of our MDR isolates will have been selected before the patient arrived. Local prophylaxis policy should therefore be informed by the local antibiogram — in our setting the combination of 61.4% ESBL prevalence and 30.7% carbapenem non-susceptibility among Enterobacterales makes routine cephalosporin prophylaxis inadequate for contaminated abdominal procedures, while indiscriminate carbapenem prophylaxis would accelerate the very resistance it seeks to circumvent.

The observed differences in length of stay (18.4 versus 11.7 days; $p < 0.001$) and the numerically higher mortality in the MDR group (6.9% versus 1.8%) are consistent in direction with the Indian mortality data of Gandra and colleagues [8], though our mortality comparison did not reach statistical significance ($p = 0.262$) and the study was not powered for this outcome.

4.6 Strengths and limitations

The principal strengths of this study are its prospective design with active CDC/NHSN-based case ascertainment rather than retrospective laboratory-record review; the application of both phenotypic and genotypic characterisation to every isolate rather than to a selected subset, which permits unbiased estimation of concordance in both directions; and the joint conduct by the Departments of Microbiology and Surgery, which allowed perioperative variables to be recorded contemporaneously rather than abstracted afterwards.

Several limitations qualify the findings. First, this is a single-centre study, and the resistance profile of one tertiary institute in a Union Territory cannot be generalised to India as a whole. Second, our PCR panel was closed: eleven determinants were targeted, and ESBLs outside the blaCTX-M, blaTEM and blaSHV families, plasmid-mediated AmpC enzymes, and non-enzymatic mechanisms such as porin loss and efflux upregulation were not assessed. This certainly underestimates the true genetic basis of resistance and accounts for at least part of the observed

discordance. Third, we did not perform molecular typing, plasmid characterisation or whole-genome sequencing, so we cannot distinguish clonal dissemination from independent acquisition, nor establish whether the blaNDM and blaOXA-48 determinants were plasmid-borne and transferable — a question of direct infection-control relevance. Fourth, the analysis of factors associated with MDR SSI was univariate; the factors examined are correlated with one another, and multivariable logistic regression would be required to establish independent effects. Fifth, the numbers of Proteus mirabilis, Enterobacter cloacae and Enterococcus faecalis isolates were small, and species-level proportions for these organisms carry wide confidence intervals. Finally, anaerobic culture was not performed routinely, so the contribution of obligate anaerobes to polymicrobial SSI at our centre is unquantified.

Future work at this centre will address the most consequential of these gaps: plasmid replicon typing and conjugation assays on the blaNDM- and blaOXA-48-carrying isolates, to establish transferability, and multivariable modelling of risk factors in a larger cohort.

5. CONCLUSION

Post-surgical wound infections at this tertiary care institute were caused predominantly by multidrug-resistant Gram-negative bacilli, with K. pneumoniae and E. coli leading and 62.6% of all isolates meeting the criteria for multidrug resistance. The molecular basis of this resistance was dominated by blaCTX-M among ESBL producers, by blaNDM and blaOXA-48 among carbapenem-resistant Enterobacterales, and by blaOXA-23 in A. baumannii — a determinant profile that closely reproduces the pattern reported from other Indian centres and that constrains the usefulness of the newer β-lactamase inhibitor combinations.

Routine phenotypic methods showed almost perfect agreement with PCR (κ 0.903–0.939) and remain a sound operational basis for antimicrobial therapy. The value of molecular testing in this setting lies not in replacing phenotypic testing but in resolving the small number of discordant isolates — oxacillin-susceptible mecA-positive S. aureus and carbapenem-susceptible blaOXA-48 carriers — whose significance is for transmission rather than for the treatment of the individual patient. A selective molecular strategy, directed at deep and organ/space infections, at isolates with borderline carbapenem MICs, and at suspected clusters, represents a rational use of limited molecular capacity in Indian tertiary care laboratories.

For surgical practice at this institute, the combination of a 61.4% ESBL rate and 30.7% carbapenem non-susceptibility among Enterobacterales indicates that prophylaxis and empirical regimens require revision against the local antibiogram, and that the modifiable perioperative factors identified here — preoperative stay beyond 48 hours and prior antimicrobial exposure — are legitimate targets for the institutional infection prevention and antimicrobial stewardship programmes.

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Reporting guideline. This manuscript was prepared in accordance with the STROBE statement for cross-sectional studies.

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