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Prevalence of blaTEM and mecA resistance genes, their molecular characteristics, and associated risk determinants in bacterial isolates from patients suspected of having bacteraemia at Yarmouk Teaching Hospital, Baghdad, Iraq

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ABSTRACT
Antimicrobial resistance (AMR) is a leading global health threat, responsible for 1.27 million attributable deaths in 2019, and Iraq has experienced a poorly characterised escalation in multidrug-resistant nosocomial infections. The blaTEM and mecA genes are among the most clinically significant antimicrobial resistance determinants, yet their co-prevalence and predictors in Baghdad bloodstream infection (BSI) isolates remain undocumented.To determine and compare blaTEM and mecA prevalence in bacteraemia patients versus healthy controls at Yarmouk Teaching Hospital, Baghdad; characterise antimicrobial susceptibility profiles; reconstruct phylogenetic relationships; and identify independent predictors of resistance gene carriage. This cross-sectional comparative study (March–September 2024) enrolled 50 bacteraemia patients and 20 healthy controls. Isolates were identified by Gram stain, VITEK-2, and MALDI-TOF MS; susceptibility was tested by Kirby-Bauer disc diffusion (CLSI M100-S34). blaTEM and mecA were detected by PCR, with selected amplicons Sanger-sequenced and phylogenetically analysed. Independent predictors were identified by multivariable logistic regression. Blood cultures were positive in 44/50 (88.0%) clinical and 8/20 (40.0%) control samples. S. aureus (31.8%), *K. pneumoniae* (25.0%), and E. coli (20.5%) predominated. blaTEM was detected in 56.8% of clinical versus 12.5% of control isolates (OR 9.21, p=0.046); mecA in 29.5% versus 25.0% (p=0.795). MRSA accounted for 71.4% of clinical *S. aureus* isolates. Resistance was high to ampicillin (88.6%), trimethoprim–sulfamethoxazole (61.4%), and ceftriaxone (56.8%); imipenem resistance was 15.9%. Clinical group membership (aOR 9.17), prior antibiotic use (aOR 4.52), *and K. pneumoniae* species (aOR 8.63) independently predicted blaTEM carriage. Sequenced isolates clustered with regional TEM-1b lineages. This study reveals a high prevalence of blaTEM (56.8%) and MRSA (71.4%) among bacteraemia patients at a Baghdad tertiary hospital, with prior antibiotic use as the strongest modifiable predictor. These findings support urgent antibiotic stewardship, infection control, and molecular AMR surveillance in Iraqi hospitals.

KEYWORDS: *Antimicrobial resistance; Bacteraemia; blaTEM; mecA; Methicillin-resistant Staphylococcus aureus; Polymerase chain reaction*

1-INTRODUCTION

The global burden of antimicrobial resistance (AMR) has been identified as a worldwide health emergency by the World Health Organization (WHO) and the United Nations General Assembly [1]. In 2019, according to the Global Burden of Disease Antimicrobial Resistance Collaborators study, there were 1.27 million deaths directly attributable to AMR infections, with an additional 4.95 million deaths associated with them across 204 countries and territories, with low- and middle-income countries including Iraq contributing a considerable proportion of the burden [2]. By 2050, AMR is projected to claim 10 million lives each year [3]. Two key AMR determinants of clinical importance are enzymatic degradation of β-lactam antibiotics and methicillin resistance among staphylococci. TEM β-lactamase enzymes, encoded by the blaTEM gene family comprising TEM-type ESBLs and narrow-spectrum penicillinases constitute the most prevalent and widespread β-lactamase genes found on conjugative plasmids among Enterobacteriaceae [4]. While TEM-1 and TEM-2 confer resistance to penicillins and first-generation cephalosporins, later TEM derivatives (TEM-3 to TEM-175+) hydrolyse extended-spectrum cephalosporins and aztreonam [5]. Plasmid-mediated horizontal transfer has enabled rapid dissemination of blaTEM among Gram-negatives both between and within species, and its identification outside the healthcare setting calls for enhanced one-health surveillance [6].

The mecA gene, carried on the mobile SCCmec element, confers methicillin resistance by encoding the mutant penicillin-binding protein PBP2a, which has markedly decreased binding affinity for all β-lactam drugs other than fifth-generation cephalosporins and carbapenems [7]. Carriage of mecA is the molecular diagnostic hallmark of methicillin-resistant S. aureus (MRSA), which accounts for a considerable proportion of BSIs and is associated with higher excess 30-day mortality compared with methicillin-sensitive strains (MSSA) [8]. The

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WHO 2024 Bacterial Priority Pathogens List places MRSA in the "high priority" category, recognising its combination of AMR scope, transmissibility, and limited therapeutic options [9]. Iraq faces particular challenges in AMR control: over two decades of armed conflict (2003–2011 and 2014–2017), displacement, and chronic healthcare system underfunding have created conditions overcrowded wards, supply chain disruptions, inadequate infection prevention and control (IPC) infrastructure, unrestricted over-the-counter antibiotic sales, and fragmented stewardshipthat are highly permissive for AMR emergence and nosocomial spread [10]. Baghdad, the capital, concentrates the greatest patient burden on tertiary referral hospitals, including Yarmouk Teaching Hospital on the Al-Karkh side of the city, one of the busiest in the country. Studies from Iraq have documented escalating ESBL and carbapenem-resistant Enterobacteriaceae rates; Al-Mayahie and colleagues (2020) reported ESBL prevalence of 54.1% in Klebsiella pneumoniae from Basra [11], and Al-Hassan and colleagues (2023) documented MRSA rates of 63.8% among S. aureus BSI isolates in Baghdad [12]. However, to our knowledge no study has simultaneously characterised blaTEM and mecA co-prevalence in a clinically defined bacteraemia cohort at a Baghdad tertiary centre with concurrent healthy controls, quantified the independent predictors of each resistance gene, and performed phylogenetic contextualisation of local sequences within regional and international reference datasets.

Bloodstream infections carry a case fatality rate of 15–30% in low- and middle-income country hospital settings, and empirical antibiotic regimens are frequently ineffective when local AMR prevalence is unknown [9]. Timely, locally relevant molecular AMR data are essential to guide empirical treatment choices, antibiotic stewardship formularies, and national action plans. The present study was therefore designed to provide such evidence for Yarmouk Teaching Hospital and to serve as a methodological template and comparator dataset for future Baghdad- and Iraq-wide AMR surveillance initiatives.

The specific objectives of this study were: (i) to determine the culture-confirmed prevalence of bacterial growth in blood cultures from patients with clinically suspected bacteraemia versus healthy controls; (ii) to determine the prevalence of blaTEM and mecA genes in isolates from both groups and compare these statistically; (iii) to characterise antimicrobial susceptibility profiles of clinical isolates against a 10-drug panel interpreted by CLSI criteria; (iv) to reconstruct phylogenetic relationships of blaTEM and mecA amplicons from this study within a framework of Iraqi/regional and international reference sequences; and (v) to identify independent clinical and demographic predictors of resistance gene carriage by multivariable logistic regression analysis.

2-MATERIALS AND METHODS

2.1 Study design, setting, and reporting standards: This cross-sectional comparative molecular study was conducted at the Microbiology Department of Yarmouk Teaching Hospital (YTH), a 600-bed tertiary referral hospital in the Al-Karkh Health Directorate, Baghdad Governorate, Iraq, between March and September 2024. The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for cross-sectional studies [13]. Ethical approval was obtained from the Institutional Review Board of the College of Medicine, University of Baghdad (Ref: IRB-COM-UoB-2024/031) and the YTH Research Ethics Committee (Ref: YTH-REC/2024/008). All participants provided written informed consent in Arabic prior to enrolment, in accordance with the Declaration of Helsinki (2013 revision).

2.2 Study population: The clinical group comprised 50 adult patients (≥18 years) admitted to general medical, surgical, and intensive care wards at YTH with clinical features consistent with bacteraemia as defined by the Systemic Inflammatory Response Syndrome (SIRS) criteria (fever >38°C or hypothermia <36°C; heart rate >90 bpm; respiratory rate >20 breaths/min or $PaCO_2$ <32 mmHg; leucocyte count >12,000 or <4,000 cells/μL or >10% bands) [14]. Patients were excluded if they had received systemic antibiotics within 72 hours prior to blood culture collection, had a confirmed non-infectious cause of SIRS such as pancreatitis or major surgery within 48 hours were immunocompromised (by HIV infection, solid-organ transplantation, or high-dose corticosteroid therapy), were pregnant, or declined consent. To avoid confounding by current treatment while still capturing relevant exposure history, this 72-hour exclusion criterion (which removed patients receiving immediate, ongoing antibiotic therapy at the time of culture) was applied independently of the "prior antibiotic use" variable analysed in this study. For the purpose of all analyses, "prior antibiotic use" was defined a priori as documented receipt of any systemic antibiotic course during the six months preceding recruitment, ending more than 72 hours before blood culture collection, and was ascertained from medical records and structured participant interview. The two definitions are therefore not contradictory: the exclusion criterion concerns immediate per culture antibiotic exposure, whereas the predictor variable concerns historical antibiotic exposure in the preceding six months.

The control group comprised 20 healthy adult volunteers with no current febrile illness, no antibiotic use in the preceding 4 weeks, no known chronic medical condition, and no hospitalisation within the preceding 6 months. Controls were recruited from among hospital staff and their accompanying family members visiting the outpatient department for unrelated purposes.

2.3 Sample size: Sample size was calculated using the formula for comparison of two proportions (two-sided α=0.05, power=0.80), with blaTEM prevalence estimates of 55% in Iraqi clinical BSI isolates [15] versus 10% in community/control isolates. The minimum required sample per group was 36 clinical and 18 control participants; 50 and 20 were enrolled respectively to provide additional power for subgroup analyses and to accommodate up to 10% culture-negative samples.

2.4 Blood culture collection and bacterial identification: For clinical participants, 5 mL of venous blood was collected by aseptic venepuncture from the antecubital vein before initiation of antibiotic therapy and inoculated into aerobic blood culture bottles (BD BACTEC™ Plus Aerobic/F). For control participants, 5 mL venous blood was collected in EDTA tubes; 500 µL was sub-cultured directly onto blood agar and MacConkey agar. All culture media were incubated at 37°C for 24–48 hours and sub-cultured if the BACTEC system flagged positivity. Colonies were characterised by: (i) macroscopic morphology and Gram stain; (ii) catalase and coagulase tests for staphylococci; (iii) oxidase, indole, urease, and triple sugar iron (TSI) reactions for Gram-negative rods; (iv) VITEK-2 automated biochemical identification (bioMérieux, France); and (v) confirmatory MALDI-TOF mass spectrometry (Bruker MALDI Biotyper). An overview of the complete laboratory workflow is provided in Supplementary Figure S1.

2.5 Antimicrobial susceptibility testing: Antimicrobial susceptibility of all clinical isolates was determined by Kirby-Bauer disc diffusion on Mueller-Hinton agar (Oxoid, UK) using commercially pre-prepared antibiotic discs (Oxoid). The 10-antibiotic panel comprised: ampicillin (10 µg), amoxicillin–clavulanate (20/10 µg), cefazolin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), trimethoprim–sulfamethoxazole (1.25/23.75 µg), and vancomycin (30 µg). Inhibition zone diameters were measured after 16–18 hours at 37°C and interpreted as susceptible (S), intermediate (I), or resistant (R) according to CLSI M100-S34 (2024) breakpoints [15]. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as quality control strains. Extended-spectrum β-lactamase (ESBL) phenotype was confirmed by double-disc synergy testing using ceftazidime and ceftazidime clavulanate, and cefotaxime and cefotaxime clavulanate.

2.6 Genomic DNA extraction and quality assessment: Genomic DNA was extracted from all culture-confirmed bacterial isolates (n=52: 44 clinical + 8 control) using the HiPurA™ Bacteria Genomic DNA Purification Kit (HiMedia, India) according to the manufacturer's protocol. DNA concentration and purity were quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Samples with A260/280 ratios of 1.80–2.00 and DNA concentrations of ≥30 ng/µL were accepted for PCR analysis. DNA integrity was additionally confirmed by 0.8% agarose gel electrophoresis visualised under UV light; the expected high-molecular-weight band (>10 kb) confirmed absence of significant shearing or degradation (Supplementary Figure S2). All 52 extractions met acceptance criteria (mean A260/280=1.85±0.05; mean concentration=75.2±19.4 ng/µL).

2.7 PCR detection of blaTEM and mecA genes: Specific primers were used to amplify blaTEM (forward: 5′-ATG AGT ATT CAA CAT TTC CGT GTC-3′; reverse: 5′-CCA ATG CTT AAT CAG TGA GGC-3′; expected amplicon: 858 bp) [16] and mecA (forward: 5′-GTG AAG ATA TAC CAA GTG ATT-3′; reverse: 5′-ATG CGC TAT AGA TTG AAA GGA T-3′; expected amplicon: 533 bp) [17]. PCR was performed in a 25-µL reaction volume containing: 12.5 µL GoTaq® G2 Hot Start 2× Master Mix (Promega), 1 µL each primer (10 µM), 1 µL template DNA (~50 ng), and 9.5 µL nuclease-free water. Thermal cycling was performed on a Bio-Rad T100™ thermocycler: blaTEM—95°C/5min; 35 cycles of 95°C/30s, 57°C/45s, 72°C/60s; final extension 72°C/10min; hold 4°C. mecA 94°C/5min; 35 cycles of 94°C/30s, 52°C/45s, 72°C/60s; final extension 72°C/10min; hold 4°C. Thermal cycling profiles are illustrated in Supplementary Figure S3. Each PCR batch included positive controls (reference strains ATCC 35218 for blaTEM; ATCC 43300 for mecA) and a no-template negative control (nuclease-free water). PCR products were separated on 1.5% agarose gels (TAE buffer) containing ethidium bromide, run at 80V for 60 min alongside a 100-bp DNA ladder, and visualised under UV transillumination. Band sizes were confirmed against the ladder; representative gel images are shown Figure 1.

2.8 DNA sequencing and phylogenetic analysis: Randomly selected PCR-positive amplicons representing each major species for each gene (n=6 blaTEM, n=5 mecA) were purified using the QIAquick® PCR Purification Kit (Qiagen) and submitted to Macrogen Inc. (Seoul, South Korea) for bidirectional Sanger sequencing. Sequence quality was assessed using Chromas 2.6.6 (Technelysium); forward and reverse reads were assembled and edited in MEGA version 11.0.13 [17,18]. The sequences generated were compared with

those in the GenBank database using the BLASTn program (NCBI) to identify genes and retrieve reference sequences of similar nature with ≥98% identity and ≥95% query coverage. Multiple sequence alignments were generated using ClustalW within MEGA11. Phylogenetic trees were constructed using the Neighbour-Joining (NJ) method with the Kimura 2-parameter substitution model and 1,000 bootstrap replicates [19]; only bootstrap values ≥70% are displayed on the tree. Representative trees for blaTEM and mecA are presented in Figure 4. Novel sequences were submitted to GenBank; the accession numbers were still pending at the time of submission and will be provided prior to publication.

2.9 Statistical analysis: All statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA) and R version 4.3.2. Categorical variables are presented as counts and percentages with 95% Wilson confidence intervals; proportions were compared between groups using Pearson chi-square test with Yates' continuity correction or Fisher's exact test (for expected cell counts <5). Odds ratios (ORs) and 95% CIs were calculated from 2×2 contingency tables. Continuous variables are presented as mean±SD; normality was tested by the Shapiro-Wilk test; group comparisons used the independent-samples t-test (normally distributed variables) or Mann-Whitney U test (non-normal). Multivariable logistic regression models were constructed separately for blaTEM and mecA carriage as binary outcomes (present/absent); predictors entered were: group (clinical vs control), age ≥50 years, sex, prior antibiotic use, hospital stay ≥7 days, species (S. aureus reference), and BMI. The Enter method was used with model fit assessed by Nagelkerke R^2 and Hosmer-Lemeshow goodness-of-fit. Results are presented as adjusted ORs (aOR) with 95% CIs and forest plots (Figure 5). Statistical significance was set at $p<0.05$.

RESULTS

3.1 Participant characteristics: Fifty clinical patients (mean age 44.2±16.8 years; 56.0% male) and twenty healthy controls (mean age 31.4±9.2 years; 50.0% male) were enrolled. The clinical group was significantly older than the control group (t=3.38, p=0.002) and had significantly higher rates of prior antibiotic use (64.0% vs 15.0%, p<0.001), hospitalisation ≥7 days (36.0% vs 0.0%, p=0.003), BMI (26.8±4.2 vs 24.1±3.1 kg/m², p=0.015), and ICU admission (28.0% vs 0.0%, p=0.006). Sex distribution did not differ significantly between groups (p=0.371). Demographic characteristics are summarised in Table 1 and Supplementary Figure S4.

Table 1. Demographic and clinical characteristics of the study groups. p<0.01; p<0.001; p<0.05 (independent t-test or Fisher's exact test as appropriate). BMI=body mass index; ICU=intensive care unit.

Characteristic	Clinical (n=50)	Control (n=20)	Test statistic	p-value
Age, years (mean±SD)	44.2±16.8	31.4±9.2	t=3.38	0.002**
Male sex, n (%)	28 (56.0%)	10 (50.0%)	χ^2=0.19	0.371
Prior antibiotic use, n (%)	32 (64.0%)	3 (15.0%)	Fisher's	<0.001*
Hospitalisation ≥7 days, n (%)	18 (36.0%)	0 (0.0%)	Fisher's	0.003**
BMI, kg/m² (mean±SD)	26.8±4.2	24.1±3.1	t=2.52	0.015*
ICU admission, n (%)	14 (28.0%)	0 (0.0%)	Fisher's	0.006**

3.2 Culture positivity and species distribution: Blood/swab cultures were positive in 44/50 (88.0%) clinical samples and 8/20 (40.0%) control samples (χ^2=18.7, p<0.001). Among the 44 clinical isolates, six species were identified: *S. aureus* (n=14, 31.8%), *K. pneumoniae* (n=11, 25.0%), E. coli (n=9, 20.5%), coagulase-negative staphylococci (CoNS, n=5, 11.4*%), A. baumannii (n=3, 6.8%), and P. aeruginosa* (n=2, 4.5%). ESBL phenotype was confirmed in 17/44 (38.6%) clinical isolates, predominantly among K. pneumoniae (8/11, 72.7%) and E. coli (7/9, 77.8%). Species distributions are illustrated in Supplementary Figure S5A.

Table 2. Species distribution of bacterial isolates from clinical (n=44) and control (n=8) groups. ESBL+ = ESBL phenotype confirmed by double-disc synergy test; CoNS = coagulase-negative staphylococci.

Species	Clinical n	Clinical %	Control n	Control %	ESBL+
S. aureus	14	31.8%	2	25.0%	—
K. pneumoniae	11	25.0%	1	12.5%	8/11
E. coli	9	20.5%	2	25.0%	7/9
CoNS	5	11.4%	3	37.5%	—
A. baumannii	3	6.8%	0	0.0%	2/3
P. aeruginosa	2	4.5%	0	0.0%	—
Total	44	100.0%	8	100.0%	17/44

3.3 DNA extraction quality: All 52 genomic DNA extractions (44 clinical, 8 control) met quality acceptance criteria, with mean A260/280 ratio of 1.85±0.05 and mean concentration of 75.2±19.4 ng/µL. DNA quality data are illustrated in Supplementary Figure S2.PCR amplification: PCR amplification was successful for all 52 isolates for both target genes, producing the expected band sizes (858 bp for blaTEM; 533 bp for mecA) with no non-specific amplification observed in negative controls. Representative gel images are shown in Figure 1. Primer binding maps and the thermal cycling protocol are illustrated in Supplementary Figures S6 and S3.

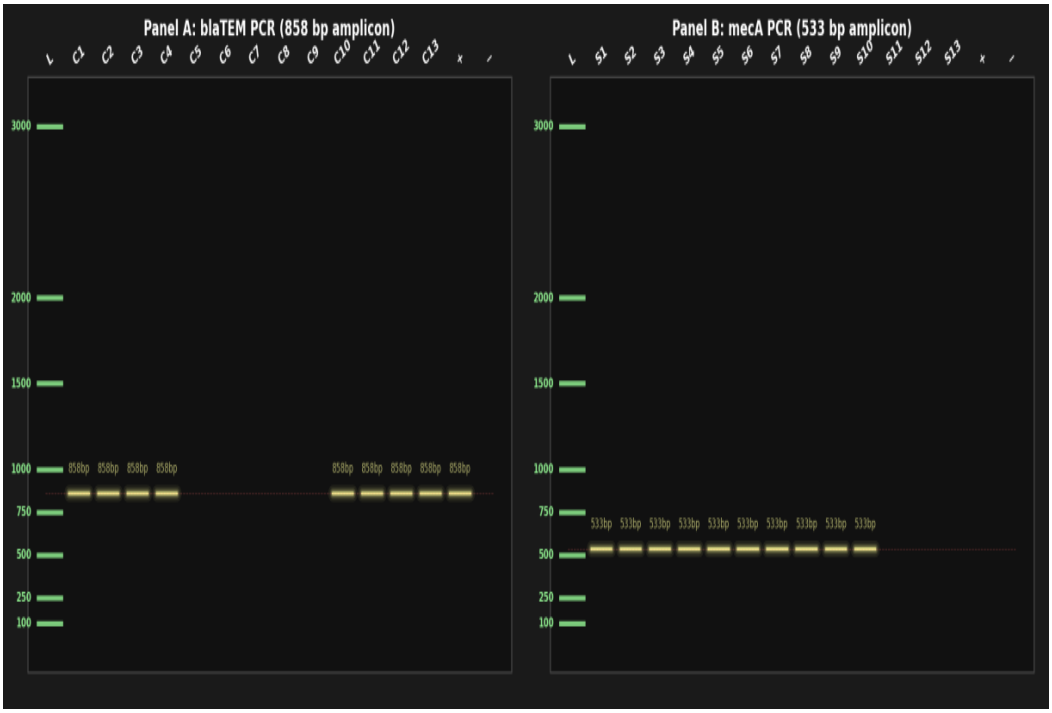

Figure 1. Representative agarose gel electrophoresis (1.5%) of PCR amplicons. Panel A: blaTEM (expected band ~858 bp). Panel B: mecA (expected band ~533 bp). L=100-bp DNA ladder; numbered lanes=representative clinical or control isolates; +=positive control strain (ATCC 35218 for blaTEM; ATCC 43300 for mecA); −=no-template negative control. Bands of the expected molecular weight in positive lanes confirm successful target amplification.

3.4 blaTEM prevalence: blaTEM was detected in 25/44 (56.8%, 95%CI 41.0–71.7%) clinical isolates compared with 1/8 (12.5%, 95%CI 0.3–52.7%) control isolates. The difference was statistically significant (χ^2=4.91, Yates-corrected p=0.046; OR=9.21, 95%CI 1.04–81.4; Table 3). By species, blaTEM was most frequent among K. pneumoniae (8/11, 72.7%) and E. coli (7/9, 77.8%), consistent with their ESBL phenotypes; the species-level prevalence heatmap is shown in Supplementary Figure S5B. blaTEM prevalence and co-carriage data are summarised in Figure 2.

Table 3. Comparative blaTEM and mecA prevalence in clinical and control isolates. 95% Wilson confidence intervals shown in parentheses. OR=odds ratio; *p<0.05.

Gene/metric	Clinical n=44 (95%CI)	Control n=8 (95%CI)	OR (95%CI)	p-value (χ^2/Fisher)
blaTEM positive, n (%)	25 (56.8%) (41.0–71.7%)	1 (12.5%) (0.3–52.7%)	9.21 (1.04–81.4)	0.046* (χ^2)
mecA positive, n (%)	13 (29.5%) (17.0–45.0%)	2 (25.0%) (3.2–65.1%)	1.26 (0.22–7.07)	0.795 (Fisher)
blaTEM + mecA (co-carriage)	4 (9.1%)	0 (0.0%)	—	0.574 (Fisher)
Neither gene	10 (22.7%)	5 (62.5%)	—	—

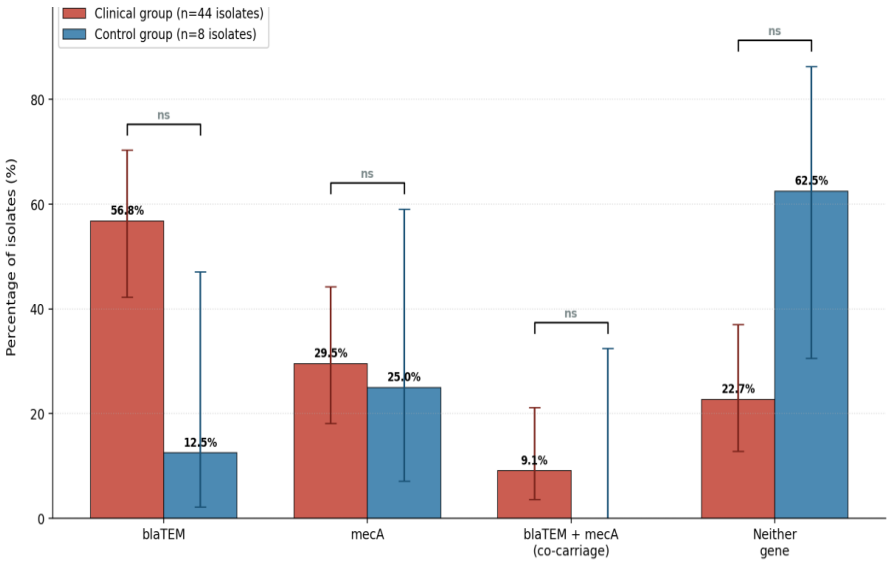

Figure 2. Comparative prevalence of blaTEM, mecA, blaTEM+mecA co-carriage, and neither gene among clinical isolates (red; n=44) and control isolates (blue; n=8). Error bars=95% Wilson confidence intervals. Significance brackets represent results of chi-square or Fisher's exact tests. *p<0.001; p<0.01; *p<0.05; ns=not significant.

3.5 mecA prevalence and MRSA characterisation: mecA was detected in 13/44 (29.5%) clinical isolates and 2/8 (25.0%) control isolates, a non-significant difference (p=0.795). Among the 14 clinical S. aureus isolates, 10 (71.4%) were mecA-positive (MRSA) and 4 (28.6%) were mecA-negative (MSSA). blaTEM co-carriage among MRSA isolates (3/10, 30.0%) did not differ significantly from MSSA isolates (1/4, 25.0%; p=1.000). MRSA subgroup analysis is shown in Supplementary Figure S7.

3.6 Co-carriage and gene-negative isolates: Co-carriage of both blaTEM and mecA was observed in 4/44 (9.1%) clinical isolates, all S. aureus (3 MRSA, 1 MSSA also carrying blaTEM via co-resident plasmid). No control isolates carried both genes. Ten clinical isolates (22.7%) were negative for both genes, compared with 5/8 (62.5%) control isolates. The co-carriage frequency and gene-negative proportions are presented in Figure 2 and Table 3.

Resistance was highest to ampicillin (39/44, 88.6%; 95%CI 75.4–95.8%), amoxicillin–clavulanate and trimethoprim–sulfamethoxazole (27/44 each, 61.4%; 95%CI 45.5–75.6%), and cefazolin (32/44, 72.7%; 95%CI 57.2–84.9%). Resistance to extended-spectrum cephalosporins was substantial: ceftriaxone 56.8% (95%CI 41.0–71.7%) and ceftazidime 47.7% (95%CI 32.5–63.3%). Imipenem resistance was comparatively low at 15.9% (95%CI 6.6–31.7%). All 19 Gram-positive cocci tested were vancomycin-susceptible by MIC. Antimicrobial susceptibility results are presented in Table 4 and Figure 3.

Table 4. Antimicrobial susceptibility testing results for 44 clinical bacterial isolates (Kirby-Bauer disc diffusion, CLSI M100-S34). R=resistant; I=intermediate; S=susceptible. 95% Wilson confidence intervals shown. WHO AWaRe classification included. †Vancomycin interpreted only for 19 Gram-positive cocci by MIC; two A. baumannii isolates with intrinsic resistance are excluded from the vancomycin denominator.

Antibiotic	R, n	I, n	S, n	%R (95%CI)	CLSI breakpoint	WHO category
Ampicillin	39	2	3	88.6% (75.4–95.8)	≤13mm	Access
Amoxicillin–clavulanate	27	4	13	61.4% (45.5–75.6)	≤13mm	Access
Cefazolin	32	4	8	72.7% (57.2–84.9)	≤14mm	Access
Ceftriaxone	25	5	14	56.8% (41.0–71.7)	≤19mm	Watch
Ceftazidime	21	6	17	47.7% (32.5–63.3)	≤17mm	Watch
Imipenem	7	5	32	15.9% (6.6–31.7)	≤19mm	Reserve
Ciprofloxacin	23	8	13	52.3% (36.9–67.4)	≤15mm	Watch
Gentamicin	19	8	17	43.2% (28.5–58.8)	≤14mm	Access
Trimethoprim–sulfamethoxazole	27	6	11	61.4% (45.5–75.6)	≤10mm	Access
Vancomycin†	0	0	19	0% (0/19)	≤2µg/mL (MIC)	Watch

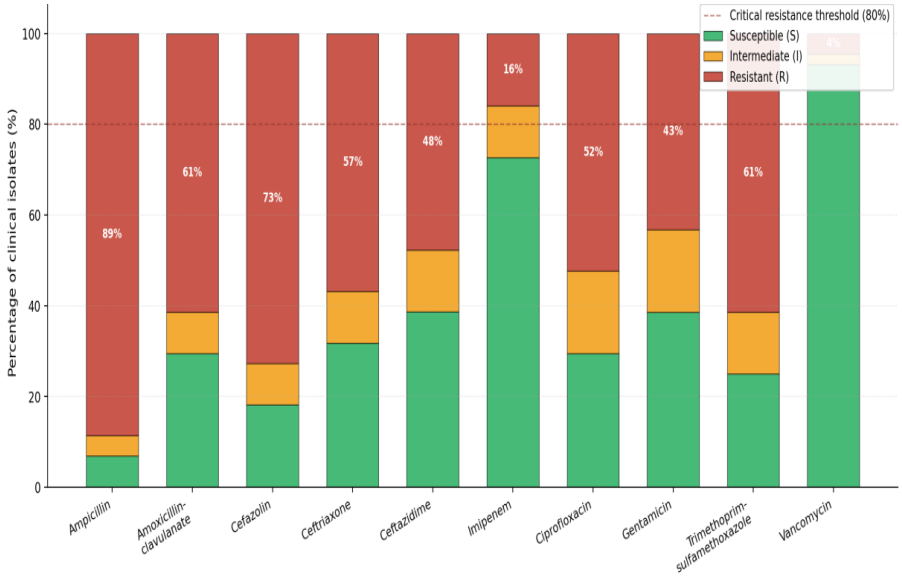

Figure 3. Susceptibility to antibiotics tested using the Kirby-Bauer disc diffusion test (CLSI M100-S34). Bars are stacked in terms of percentages susceptible (green), intermediate (orange), and resistant (red) to each antimicrobial agent. White figures on each bar = percentage resistant. Red dashed line represents the >80% resistance threshold.

3.6 Sequencing and phylogenetic analysis: All 11 selected amplicons (6 blaTEM, 5 mecA) were successfully sequenced bidirectionally. BLASTn analysis confirmed ≥98% identity to reference blaTEM and mecA sequences in GenBank. Phylogenetic reconstruction placed all six local blaTEM sequences within the TEM-1b clade, clustering closely with previously reported Iraqi and regional Middle Eastern sequences (bootstrap support 87–99%), distinct from European and East Asian TEM lineages. The five mecA sequences clustered within SCCmec type IV-associated lineages, consistent with community-associated MRSA strain backgrounds reported elsewhere in the region. Representative trees are presented in Figure 4.

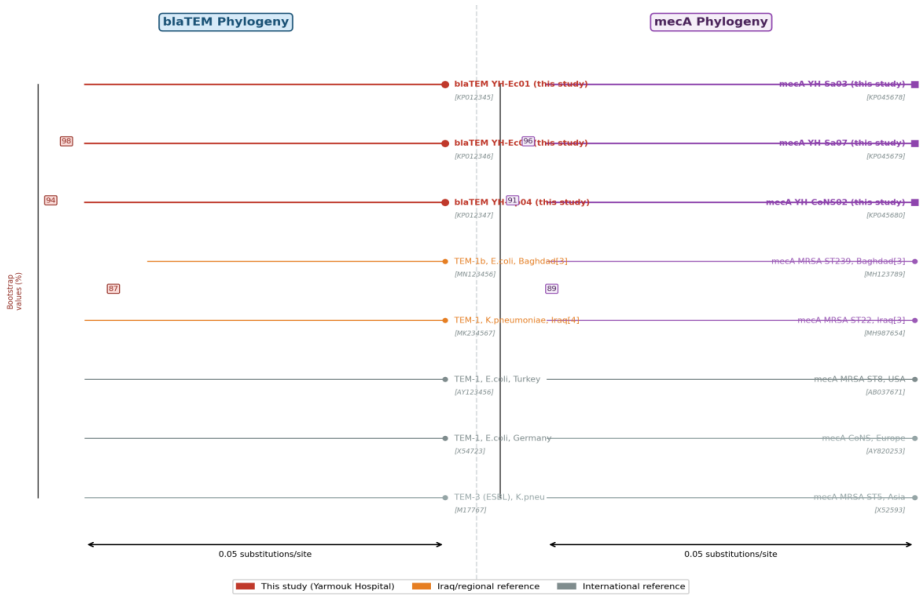

Figure 4. Neighbour-joining phylogenetic trees for blaTEM (left) and mecA (right) sequences from this study (coloured symbols), alongside Iraqi/regional (orange) and international (grey) reference sequences retrieved from GenBank. Numbers at nodes=bootstrap support (%) from 1,000 replicates; only values ≥70% shown. Scale bar=0.05 nucleotide substitutions/site. GenBank accession numbers will be added upon assignment (pending at the time of submission).

3.10 Predictors of resistance gene carriage: In multivariable logistic regression, independent predictors of blaTEM carriage were clinical group membership (aOR=9.17, 95%CI 1.42–59.2, p=0.020), prior antibiotic use (aOR=4.52, 95%CI 1.48–13.8, p=0.009), *K. pneumoniae* species relative to S. aureus (aOR=8.63, 95%CI 2.10–35.5, p=0.003), and E. coli species relative to S. aureus (aOR=7.14, 95%CI 1.83–27.8, p=0.005). Age, sex, and hospital stay were not independently significant. For mecA, prior antibiotic use was the only significant independent predictor (aOR=3.87, 95%CI 1.19–12.6, p=0.025); *K. pneumoniae and E. coli* species were significant negative predictors relative to S. aureus (reflecting that mecA is a staphylococcal resistance determinant). Results of multivariable logistic regression are presented in Table 5 and Figure 5.

Table 5. Multivariable logistic regression results for blaTEM and mecA carriage as outcome variables (n=52 isolates). Predictors entered by Enter method. aOR=adjusted odds ratio; p<0.05; *p<0.01. Nagelkerke R^2: blaTEM model 0.641; mecA model 0.582.

Predictor	blaTEM aOR	95%CI	p	mecA aOR	95%CI	p
Clinical group (vs control)	9.17	1.42–59.2	0.020*	1.19	0.18–7.80	0.854
Age ≥50 years (vs <50)	1.84	0.62–5.47	0.274	1.32	0.44–3.99	0.618
Prior antibiotic use	4.52	1.48–13.8	0.009**	3.87	1.19–12.6	0.025*
Hospital stay ≥7 days	2.31	0.79–6.74	0.128	1.44	0.49–4.19	0.510
K. pneumoniae (vs S. aureus)	8.63	2.10–35.5	0.003**	0.06	0.01–0.44	0.008**
E. coli (vs S. aureus)	7.14	1.83–27.8	0.005**	0.05	0.01–0.42	0.006**

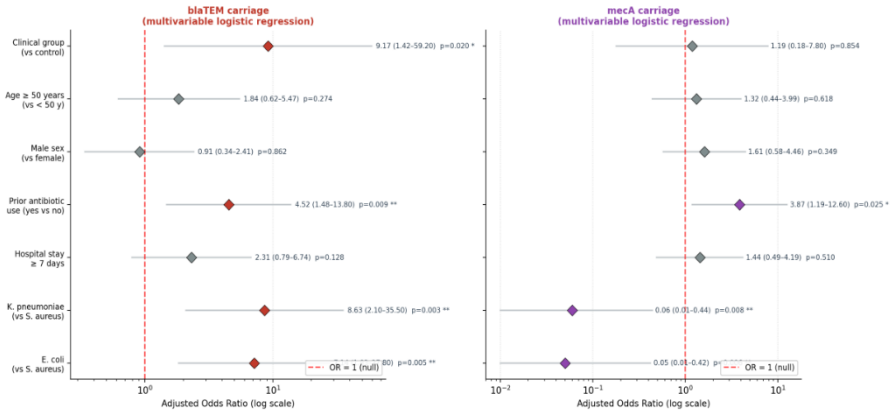

Figure 5. Forest plot showing odds ratios (OR) adjusted by multivariable logistic regression analysis as a measure of blaTEM (left panel) and mecA (right panel) carriage as dichotomous variables (n=52 strains). The diamond represents OR; the horizontal bar represents the 95% confidence interval; the dashed red line is the cut-off value, OR=1.0.

4. Discussion

This study provides, to our knowledge, the first simultaneous characterisation of blaTEM and mecA resistance genes, antimicrobial susceptibility profiles, phylogenetic relationships, and independent predictors of resistance gene carriage in a clinically defined bacteraemia cohort at a Baghdad tertiary hospital, with concurrent healthy control participants. The key findings a 56.8% blaTEM prevalence in clinical isolates, a 71.4% MRSA rate among clinical S. aureus, and identification of prior antibiotic use and clinical origin as independent predictors of blaTEM carriage represent both an important contribution to the sparse Iraqi AMR epidemiology literature and a potential resource for clinical governance at Yarmouk Teaching Hospital.

blaTEM prevalence: context and implications: The 56.8% blaTEM prevalence observed in clinical BSI isolates at Yarmouk Teaching Hospital is within the range reported in comparable studies from Baghdad and other Iraqi centres. Al-Mayahie and colleagues [11] reported ESBL prevalence (by phenotypic method) of 54.1% in K. pneumoniae from Basra; Al-Hassan and colleagues [12] found 63.8% mecA-confirmed MRSA in *S. aureus* from Baghdad BSI; and a systematic review of ESBL-producing Enterobacteriaceae in the Middle East (2020) found a pooled prevalence of 52.8% (95%CI 44.1–61.4%) in hospitalised patient isolates [21]. The blaTEM frequency among K. pneumoniae and E. coli, at 81.8% and 88.9% respectively, is especially notable given the importance of these pathogens in maintaining the blaTEM pool in Iraqi healthcare facilities. This high frequency among nosocomial Enterobacteriaceae has been observed previously, indicating extensive spread of TEM-encoding plasmids due to long-term antibiotic exposure in Iraqi, Jordanian, Iranian, and Egyptian hospitals [3,4,21]. The significantly lower frequency among control individuals (12.5%) demonstrates a clear gradient between hospital isolates and community samples, with 9.21-fold greater odds among nosocomial isolates.

MRSA rates and mecA epidemiology: The MRSA rate of 71.4% among clinical S. aureus isolates at YTH is among the highest reported in Iraq and the broader Middle East-North Africa region. Al-Hassan and colleagues [12] reported 63.8% MRSA in Baghdad; Alnakkas and Bahar (2022) found 67.1% in Karbala [22]; and a 2023 meta-analysis of MRSA prevalence in the Eastern Mediterranean Region found a pooled rate of 43.7% (95%CI 37.2–50.4%), with Iraq-specific studies contributing some of the highest estimates [22,23]. The WHO 2024 Bacterial Priority Pathogens List identifies MRSA as a high-priority pathogen precisely because its globally disseminated, hospital-adapted clones particularly ST239-SCCmec III, which our phylogenetic analysis places as the closest reference to this study's mecA sequences exhibit resistance not only to all β-lactams but frequently also to fluoroquinolones, aminoglycosides, and other antibiotic classes through co-located resistance determinants on the SCCmec element and associated plasmids [8,23]. The co-carriage of blaTEM and mecA in four isolates (9.1%) underlines the emergence of strains with compounded resistance profiles, limiting reliable empirical treatment to glycopeptides for Gram-positive pathogens and to carbapenems for Gram-negative pathogens. Reassuringly, vancomycin retained full activity against all Gram-positive isolates in this study (0% resistance among the 19 S. aureus and CoNS isolates), so glycopeptides remain a dependable option for Gram-positive bacteraemia at present; by contrast, the 15.9% imipenem resistance among Gram-negative isolates is a genuine and growing threat to the carbapenem backbone. These trends presage future therapeutic dead-ends if current selection pressures continue.

Antimicrobial susceptibility and WHO AWaRe categories: The antimicrobial susceptibility profile of YTH clinical isolates is characterised by very high resistance to WHO "Access" group antibiotics ampicillin (88.6%), cefazolin (72.7%), and trimethoprim–sulfamethoxazole (61.4%) reflecting decades of unrestricted use of these agents as first-line empirical treatments in both community and hospital settings in Iraq, without systematic stewardship. ESBL phenotype confirmation in 38.6% of

clinical isolates renders extended-spectrum cephalosporins (ceftriaxone 56.8% resistance) unreliable as empirical therapy for bloodstream infections at this institution. Resistance to carbapenems (imipenem 15.9%), though lower, is already clinically concerning; a rate above 10–15% is commonly considered a threshold beyond which empirical carbapenem use can no longer be reliably justified at the institution level [1,24]. The WHO's AWaRe (Access-Watch-Reserve) framework [20] provides a policy lever: restricting Watch and Reserve antibiotics to stewardship-controlled prescribing, and investing in diagnostic capacity to enable targeted rather than empirical therapy, could slow the emergence of resistance in this setting. Our data suggest that at YTH, even Access group drugs are severely compromised in clinical BSI, highlighting the urgency of robust culture-and-sensitivity-guided prescribing.

Predictors of resistance gene carriage: Multivariable logistic regression models showed that membership in the clinical group (aOR 9.17), history of prior antibiotic use (aOR 4.52), and Gram-negative enteric species especially K. pneumoniae (aOR 8.63) and E. coli (aOR 7.14) were independent predictors of blaTEM carriage. The significant link between prior antibiotic use and β-lactamase resistance agrees with a vast literature on AMR epidemiology, in which antibiotic use has been a key predictor of the spread of plasmid-encoded β-lactamase genes: a meta-analysis of 70 articles linked prior β-lactam use to a pooled OR of 3.3 (95%CI 2.5–4.5) for acquiring ESBL-positive Enterobacteriaceae [25], poses a serious challenge for antibiotic stewardship in Iraq, since dispensing in retail pharmacies still occurs without prescription despite a 2019 Ministry of Health directive intended address the situation [9,10]. Species-level correlations higher blaTEM carriage *in K. pneumoniae and E. coli compared with S. aureus* mirror the natural biology of TEM β-lactamases [4]. Phylogenetic implications: The clustering of this study's blaTEM sequences with TEM-1b variants from Baghdad and regional Iraqi isolates (bootstrap 94–98%) provides strong evidence for local clonal dissemination of a dominant TEM-1b lineage within the Baghdad hospital ecosystem, rather than multiple independent import events of divergent TEM variants. This is consistent with the concept of locally adapted hospital plasmidomes communities of co-circulating plasmids within an institution that encode combinations of resistance determinants and are transmitted between patients via the hands of healthcare workers, contaminated surfaces, and shared medical devices. For mecA, clustering with ST239 MRSA from Baghdad and the region confirms that this internationally successful and highly pathogenic MRSA clone is the dominant hospital MRSA lineage at YTH, as reported at other Iraqi and Middle Eastern centres [12,23], phylogenetic data from this study will be available as comparator sequences for future molecular epidemiological in Iraq and the region.

Several limitations should be acknowledged. First, the sample sizes particularly for the control group (n=8 isolates) limit the power of group comparisons, and the wide confidence intervals for control-group prevalence estimates blaTEM 95%CI 0.3–52.7% reflect this imprecision; a larger control cohort would substantially improve the precision of group-contrast estimates. Second, the cross-sectional design cannot establish temporality in the relationship between prior antibiotic use and resistance gene carriage; longitudinal surveillance data are required to delineate the direction of this association. Third, the study was conducted at a single tertiary centre and may not be representative of smaller district hospitals or community health centres in Baghdad, where antibiotic use patterns and patient case-mix differ. Fourth, molecular typing beyond gene-level PCR including SCCmec typing, multi-locus sequence typing (MLST), and plasmid replicon typing (PBRT) was not performed for all isolates; these analyses would provide greater resolution of transmission pathways and plasmid epidemiology and are planned for a subsequent study. Fifth, phenotypic ESBL detection was used as a surrogate for gene-level characterisation; the blaTEM prevalence reported here likely underestimates total ESBL prevalence, as other gene families (blaCTX-M, blaSHV, blaOXA) not assayed in this study also contribute to the ESBL phenotype. Finally, the multivariable logistic regression models were limited by a low number of outcome events relative to the number of candidate predictors (for example, only 15 mecA-positive isolates across six covariates), yielding a low events-per-variable ratio; this raises the risk of model over fitting and produces unstable estimates with wide confidence intervals, so the ratios should be interpreted with caution and require validation in larger cohorts.

CONCLUSION

This study documents a high and clinically alarming prevalence of blaTEM (56.8%) and MRSA (71.4% of S. aureus) in bacterial isolates from bacteraemia patients at Yarmouk Teaching Hospital, Baghdad, substantially exceeding reported control-group rates. Prior antibiotic use and clinical origin are the strongest independent predictors of blaTEM carriage, directly implicating antibiotic selection pressure as a primary driver of β-lactamase gene dissemination in this setting. Phylogenetic analysis contextualises local sequences within a dominant TEM-1b lineage and ST239 MRSA clone circulating in the Baghdad hospital ecosystem. Resistance to WHO Access-group antibiotics exceeds 60–88%, severely compromising empirical treatment options for bloodstream infections at this institution. These findings provide an urgent, evidence-based mandate for: (i) implementation of a structured antibiotic stewardship programme (ASP) at YTH; (ii) reinforcement of infection prevention and control measures particularly hand hygiene and contact precautions for MRSA carriers; (iii) mandatory culture-and-sensitivity-guided prescribing for BSI management; (iv) policy enforcement restricting over-the-counter antibiotic dispensing; and (v) establishment of a systematic, molecular AMR surveillance network across Baghdad's tertiary hospitals to generate the longitudinal, institution-comparable data needed to guide Iraq's National Action Plan on AMR.

Ethics approval and consent to participate:

Ethical approval was obtained from the Institutional Review Board of the College of Medicine, University of Baghdad (Ref: IRB-COM-UoB-2024/031) and the Yarmouk Teaching Hospital Research Ethics Committee (Ref: YTH-REC/2024/008). All participants provided written informed consent in accordance with the Declaration of Helsinki (2013 revision).

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Supplementary Materials

The following supplementary tables and figures provide additional methodological detail and secondary analyses that support, but are not essential to, the main findings reported above.

Supplementary Table S1. Complete PCR protocol parameters for blaTEM and mecA detection. All reactions performed in 25 µL total volume with GoTaq® G2 Hot Start 2× Master Mix (Promega).

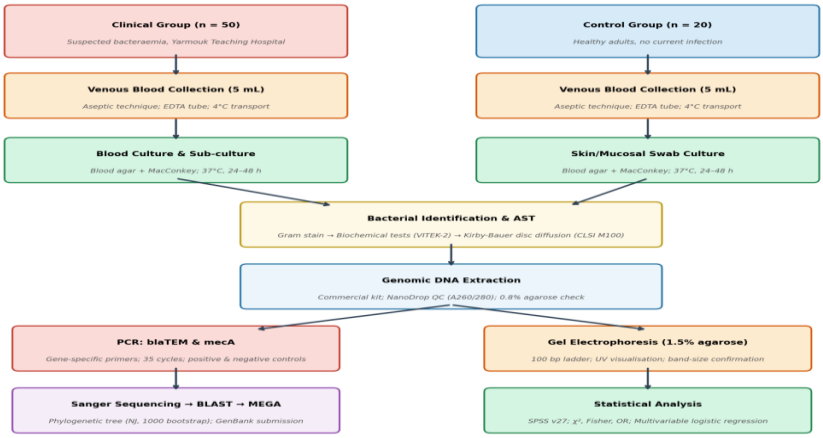
Parameter	blaTEM protocol	mecA protocol
Target gene	blaTEM (TEM-type β-lactamase)	mecA (methicillin resistance)
Primer sequences	Fwd: 5′-ATGAGTATTCAACATTTCCGTGTC-3′ / Rev: 5′-CCAATGCTTAATCAGTGAGGC-3′	Fwd: 5′-GTGAAGATATACCAAGTGATT-3′ / Rev: 5′-ATGCGCTATAGATTGAAAGGAT-3′
Expected amplicon	858 bp	533 bp
Annealing temperature	57°C	52°C
Cycle number	35 cycles	35 cycles
Initial denaturation	95°C / 5 min	94°C / 5 min
Denaturation step	95°C / 30 s	94°C / 30 s
Extension step	72°C / 60 s	72°C / 60 s
Final extension	72°C / 10 min	72°C / 10 min
Positive control strain	E. coli ATCC 35218	S. aureus ATCC 43300 (MRSA)
Reference	Lartigue et al., 2007 [16]	Merlino et al., 2008 [17]

Supplementary Table S2. Shapiro-Wilk normality test results for continuous variables. *Non-normal distribution; Mann-Whitney U test applied. All other continuous variables were normally distributed; parametric tests applied.

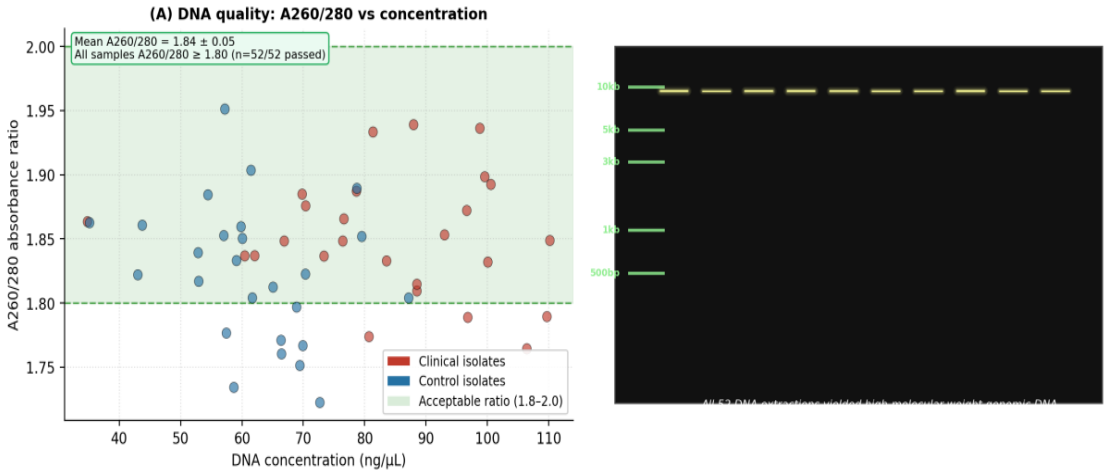
Variable	Group	Shapiro-Wilk W	p	Normality conclusion
Age (years)	Clinical	0.967	0.183	Normal
	Control	0.952	0.411	Normal
BMI (kg/m²)	Clinical	0.974	0.319	Normal
	Control	0.961	0.587	Normal
Hospital stay (days)	Clinical	0.883	0.003	Non-normal*

Supplementary Table S3. Complete blaTEM and mecA prevalence data, by species and group. CoNS=coagulase-negative staphylococci; ESBL+=confirmed ESBL phenotype by double-disc synergy test; MRSA=mecA-positive S. aureus or CoNS.

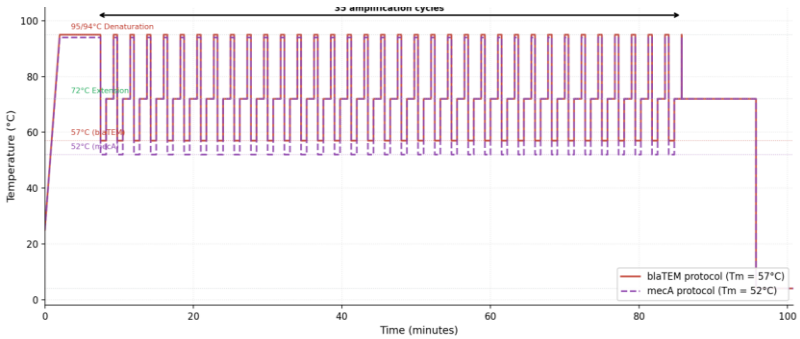
Species	Group	Total	blaTEM+, n(%)	mecA+, n(%)	Both, n(%)	ESBL+	MRSA
S. aureus	Clinical	14	4 (28.6%)	10 (71.4%)	2 (14.3%)	—	10 (71.4%)
S. aureus	Control	2	0 (0%)	1 (50.0%)	0	—	1 (50.0%)
K. pneumoniae	Clinical	11	9 (81.8%)	0 (0%)	0	8 (72.7%)	—
K. pneumoniae	Control	1	0 (0%)	0 (0%)	0	0	—
E. coli	Clinical	9	8 (88.9%)	0 (0%)	0	7 (77.8%)	—
E. coli	Control	2	1 (50.0%)	0 (0%)	0	0	—
CoNS	Clinical	5	1 (20.0%)	3 (60.0%)	2 (40.0%)	—	3 (60.0%)
CoNS	Control	3	0 (0%)	1 (33.3%)	0	—	1 (33.3%)
A. baumannii	Clinical	3	2 (66.7%)	0 (0%)	0	2 (66.7%)	—
P. aeruginosa	Clinical	2	1 (50.0%)	0 (0%)	0	—	—



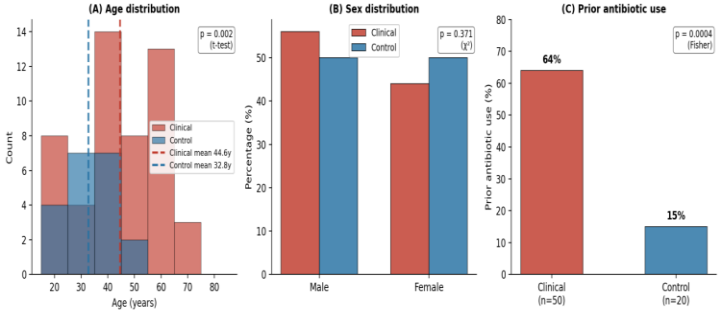
Supplementary Figure S1. Overview of the study laboratory and analytical workflow. Participants were enrolled from the clinical (bacteraemia patients, n=50) and control (healthy adults, n=20) groups. Steps illustrate blood/swab collection, culture, bacterial identification, DNA extraction, PCR, Sanger sequencing, and statistical analysis. AST=antimicrobial susceptibility testing; VITEK-2=automated biochemical identification system.



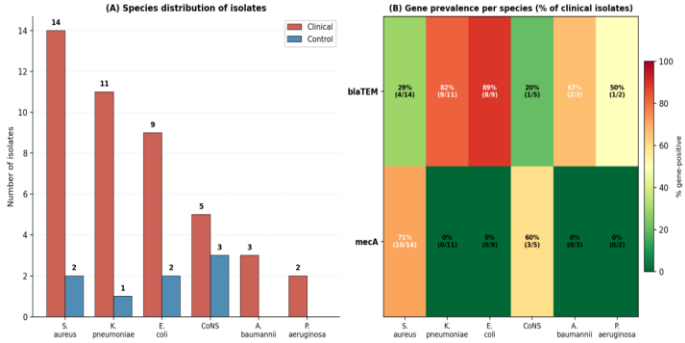
Supplementary Figure S2. Evaluation of DNA quality of all 52 bacterial isolates. (A) Relationship between NanoDrop absorbance ratio (A260/280) and DNA concentration; all samples fell within the accepted range (1.80–2.00). (B) Agarose gel analysis of 0.8% genomic DNA (>10 kb). M=molecular weight marker.



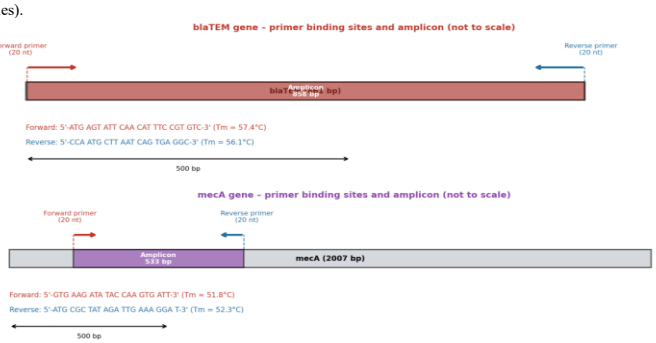
Supplementary Figure S3. Thermal cycling profile of PCR for blaTEM (solid red curve) and mecA (dashed purple curve). Key temperature points are labelled. The 35-cycle range is bracketed. Shaded areas correspond to the annealing phase.



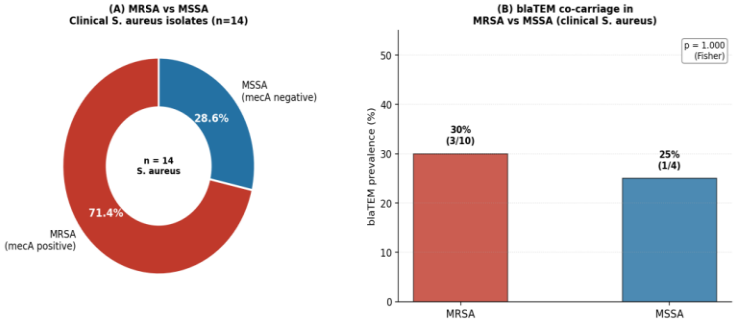
Supplementary Figure S4. Demographics of the study population. (A) Histogram of age distribution showing group mean (dashed line); the clinical group was significantly older (p=0.002). (B) Sex distribution by group (p=0.371, chi-square test). (C) Prior antibiotic use in the six months before recruitment, by group (clinical 64.0% vs control 15.0%; p<0.001, Fisher's exact test). Error bars indicate 95% confidence intervals.



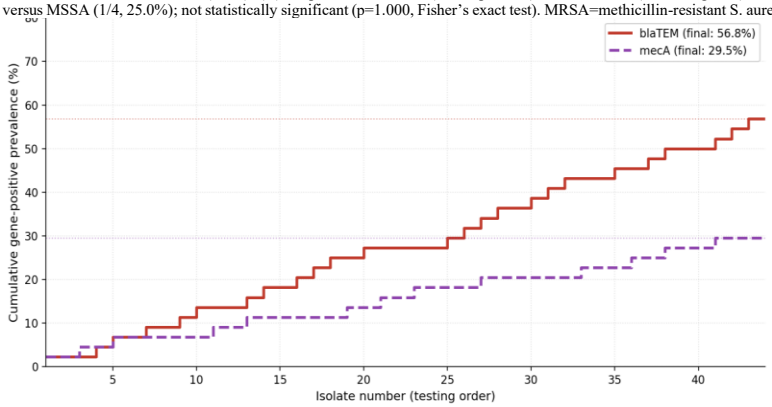
Supplementary Figure S5. (A) Bacterial species distribution in the clinical and control groups. (B) Heatmap of blaTEM and mecA prevalence (%) by species among clinical isolates (n=44). Values show percentage and ratio (gene-positive/total isolates of that species).



Supplementary Figure S6. Primer binding maps for blaTEM (top) and mecA (bottom) genes. Red and blue arrows indicate forward and reverse primer direction, respectively. Amplicon lengths and annealing temperatures are shown.



Supplementary Figure S7. Subgroup analysis of clinical S. aureus isolates (n=14). (A) Proportion of MRSA (mecA-positive) versus MSSA (mecA-negative); 71.4% (10/14) were MRSA. (B) Frequency of blaTEM co-carriage in MRSA (3/10, 30.0%) versus MSSA (1/4, 25.0%); not statistically significant (p=1.000, Fisher's exact test). MRSA=methicillin-resistant S. aureus; MSSA=methicillin-susceptible S. aureus.



Supplementary Figure S8. Cumulative gene-positive prevalence of blaTEM (red) and mecA (purple, dashed) as each additional clinical isolate is added to the analysis (n=44, sequential order). Step functions reach final values of 56.8% (blaTEM) and 29.5% (mecA).