SUPPLEMENTARY MATERIAL

Supplementary Methods S1. Detailed methodological information

S1.1. Study setting

The study site is a tertiary-level referral center in the province of Konya, Central Anatolia Region of Türkiye, with approximately 1,000 inpatient beds, including 100 intensive care unit beds. The hospital provides secondary- and tertiary-level care to the population of the province and receives referrals from surrounding provinces; its medical microbiology laboratory serves all inpatient and outpatient units of the institution.

S1.2. Isolate selection and exclusion criteria

Only non-replicate isolates were analysed. When multiple positive cultures were obtained from the same patient from different clinical specimens (different anatomical sites or subsequent episodes), only the first chronologically isolated strain was included regardless of specimen type, and all subsequent isolates from the same patient were excluded. Isolates recovered from clinical specimens submitted for diagnostic purposes were included, whereas specimens submitted as part of routine active surveillance screening (e.g., nasal screening cultures obtained for MRSA colonization surveillance) were excluded; the nasal cultures included in Table 1 of the main manuscript were therefore specimens submitted for diagnostic evaluation. Because clinical criteria for infection were not available, the included isolates are interpreted as isolates recovered from diagnostic clinical specimens rather than as confirmed indicators of active infection.

A total of 912 *S. aureus* isolates were retrieved from the laboratory information system and screened for eligibility. The following were excluded: (i) repeat isolates from patients already represented in the cohort; (ii) specimens submitted for active surveillance screening; (iii) records with incomplete data; (iv) isolates without an interpretable oxacillin result; and (v) specimens dated outside the study period. The final analytical cohort comprised 391 distinct isolates, one per patient (Figure 1 of the main manuscript).

S1.3. Microbiological identification

Clinical specimens were inoculated onto 5% sheep blood agar and esculin methylene blue agar and incubated at 35-37 °C for 18-24 hours under aerobic conditions. Colonies were evaluated for morphology, haemolysis, and pigment formation. Suspicious colonies were Gram-stained to confirm Gram-positive cocci in grape-like clusters, and catalase-positive isolates were identified as *S. aureus* by the coagulase test. Mannitol fermentation on mannitol salt agar, the DNase test, and the thermonuclease test were used when necessary to support identification.

S1.4. Harmonization of susceptibility interpretation

The software of the BD Phoenix™ system's automated system is designed annually to incorporate the current EUCAST clinical breakpoints; isolates were therefore initially interpreted according to the contemporary EUCAST versions (v11.0 to v15.0). All categorical results were subsequently cross-verified against the EUCAST Clinical Breakpoint Tables version 16.0 (valid from 2026) to ensure consistency. Because quantitative minimum inhibitory concentration (MIC) values were archived in the laboratory information system for oxacillin only, this cross-verification was performed at the level of the categorical result and was restricted to agents whose clinical breakpoints did not change between EUCAST v11.0 and v15.0. Agents whose breakpoints were revised within or after this interval, most notably trimethoprim-sulfamethoxazole (susceptibility breakpoint lowered in EUCAST v16.0, 2026), could not be re-categorized retrospectively.

Methicillin resistance was determined qualitatively with the integrated cefoxitin-based screening and oxacillin algorithms of the BD Phoenix system, and the expert software categorized isolates as MRSA or MSSA. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were processed alongside clinical isolates for quality control. Throughout the study period, every result for the oxacillin/cefoxitin panel was reported as susceptible or resistant; no isolate was reported in the "susceptible, increased exposure" category, and no re-coding or exclusion was required on this basis.

S1.5. Completeness of susceptibility data

Completeness was verified before analysis. Interpretable results for all twelve antibiotic agents shown in Table 2 of the main manuscript were available for all 391 isolates (100%), and methicillin resistance was determined for every isolate. Penicillin was not part of the standard reporting panel throughout the study period; a penicillin result was available for 345 isolates (88.2%).

S1.6. Multiple antibiotic resistance index

The MAR index is an agent-based measure (the proportion of individual antimicrobial agents to which an isolate is resistant), whereas the MDR, XDR and PDR classification is based on antimicrobial categories. Oxacillin was excluded from the denominator of the MAR index because, in *S. aureus*, oxacillin resistance is the phenotypic marker of methicillin resistance and represents the beta-lactam category already represented by penicillin; retaining both agents would count the same category twice. The index was therefore calculated over 13 antibiotic agents: the 12 shown in Table 3 with a penicillin result and over 12 antimicrobial agents for the 46 isolates without one. The 0.2 threshold was originally proposed for assessing antibiotic exposure in bacteria from sources with high antibiotic use than those with low exposure, in a setting in which exposure was presumed. Because individual prescribing data, hospital antimicrobial consumption, and stewardship indicators were not available in the present study, the index was therefore used only as a descriptive measure of the accumulated resistance burden, and no inference is made about antimicrobial exposure or selective pressure.

S1.7. Additional statistical details

Normality of continuous variables was assessed visually (histograms and Q-Q plots) and with the Kolmogorov-Smirnov and Shapiro-Wilk tests. The relationship between patient age and the MAR index was examined with Spearman's rank correlation coefficient. The 95% confidence intervals of annual resistance proportions were calculated with the Wilson score method. For TMP-SMX, heterogeneity between individual years was additionally examined with Pearson's chi-squared test. For pairwise co-resistance, odds ratios and 95% confidence intervals were calculated with the logit (Woolf) method; when a 2×2 table contained a zero cell, the odds ratio was not estimated, and only the phi coefficient and Fisher's exact test are reported. The multivariable logistic models, calendar year was entered as an ordinal variable, so that adjusted odds ratios express the change per 1-year increase. The pairwise co-resistance analysis comprised 28 agent pairs within each of the MRSA and MSSA groups; because no adjustment for multiple comparisons was made, these analyses are regarded as exploratory and hypothesis-generating.

Supplementary Results S1. Secondary analyses

Temporal trends

Trimethoprim-sulfamethoxazole (TMP-SMX) resistance varied non-monotonically across the study period (5.6%, 6.8%, 8.2%, 1.0% and 7.8% from 2021 to 2025); neither the test for trend (p=0.878) nor a test for heterogeneity between individual years (Pearson's chi-squared test, p=0.199) was significant. In the multivariable models, calendar year was not independently associated with levofloxacin (aOR 1.08, 95% CI 0.78-1.48, p=0.648) or TMP-SMX resistance (aOR 0.78, 95% CI 0.54-1.14, p=0.207), suggesting that the crude changes in these agents were largely accounted for by the increasing proportion of MRSA isolates over time.

Age and resistance

Among MRSA isolates, resistance to individual agents did not differ significantly between age groups for any agent. No correlation was found between patient age and the MAR index (Spearman's rho=0.008; p=0.870).

MAR index and resistance phenotypes

The highest mean MAR index values were observed in intensive care units and surgical units (0.144 in both; Supplementary Table S2), but differences between clinical units were not significant (Kruskal-Wallis H=2.771; p=0.597). The maximum number of antimicrobial categories to which any single isolate was resistant was eight of the eleven categories represented on the panel; therefore, no isolate met the XDR or PDR criteria. All isolates remained susceptible to vancomycin and to the oxazolidinone and amikacin+ciprofloxacin, and glycopeptide resistance was confined to the three teicoplanin-resistant isolates (0.8%).

Co-resistance

Among MRSA isolates, 95.2% (20/21) of clindamycin-resistant isolates were also resistant to erythromycin, 61.5% (8/13) of gentamicin-resistant isolates were also resistant to clindamycin, and 47.8% (11/23) of tetracycline-resistant isolates were also resistant to clindamycin. Among MSSA isolates, 93.9% (46/49) of clindamycin-resistant isolates were also resistant to erythromycin. The association between erythromycin and fusidic acid resistance was not nominally significant in MSSA (3.5%, 95% CI 1.28-7.78; p=0.014) but not in MSSA isolates (OR 3.43, 95% CI 0.93-12.64; p=0.072). The only inverse association was between erythromycin and TMP-SMX resistance in MRSA isolates (φ=-0.23); given the number of pairwise comparisons, this finding, like the other pairwise associations, is regarded as exploratory. All pairwise statistics are given in Supplementary Table S3.

Supplementary Table S1. Annual numbers and rates of resistant *Staphylococcus aureus* isolates (MRSA and MSSA combined), 2021-2025

Agent	2021 (n=54)	2022 (n=73)	2023 (n=61)	2024 (n=101)	2025 (n=102)	Total (n=391)	P for trend
Ciprofloxacin	4 (7.4) [2.0-17.6]	3 (4.1) [1.4-11.4]	8 (13.1) [6.8-23.8]	11 (10.9) [6.2-18.5]	27 (26.5) [18.9-35.8]	53 (13.6) [10.5-17.3]	<0.001
Erythromycin	8 (14.8) [7.7-26.6]	11 (15.1) [8.6-25.0]	15 (24.6) [15.5-36.7]	26 (25.7) [18.2-35.5]	29 (28.4) [20.6-37.8]	89 (22.8) [18.9-27.2]	0.013
Clindamycin	7 (13.0) [6.4-24.4]	9 (12.3) [6.6-21.8]	11 (18.0) [10.4-29.5]	17 (16.8) [10.8-25.3]	26 (25.5) [18.0-34.7]	70 (17.9) [14.4-22.0]	0.024
Fusidic acid	2 (3.7) [1.0-12.5]	6 (8.2) [3.8-16.8]	7 (11.5) [5.7-21.8]	9 (8.9) [4.8-16.1]	16 (15.7) [9.9-24.0]	40 (10.2) [7.6-13.6]	0.028
Levofloxacin	4 (7.4) [2.9-17.6]	3 (4.1) [1.4-11.4]	8 (13.1) [6.8-23.8]	6 (5.9) [2.8-12.4]	15 (14.7) [9.1-22.9]	36 (9.2) [6.7-12.5]	0.088
Tetracycline	4 (7.4) [2.9-17.6]	6 (8.2) [3.8-16.8]	3 (4.9) [1.7-13.5]	12 (11.9) [6.9-19.6]	13 (12.7) [7.6-20.6]	38 (9.7) [7.2-13.1]	0.143
Gentamicin	4 (7.4) [2.9-17.6]	3 (4.1) [1.4-11.4]	4 (6.6) [2.6-15.9]	10 (9.9) [5.4-17.9]	12 (11.8) [6.9-19.6]	33 (8.4) [6.0-11.7]	0.307
TMP-SMX	3 (5.6) [1.9-15.1]	5 (6.8) [3.0-15.1]	5 (8.2) [3.6-17.8]	1 (1.0) [0.2-5.4]	8 (7.8) [4.0-14.7]	22 (5.6) [3.7-8.4]	0.878

Values are the number of resistant isolates (%) [95% confidence interval, Wilson method]. P-values were obtained with the chi-squared test for trend (Cochran-Armitage). No isolate was resistant to vancomycin, linezolid or rifampicin; teicoplanin resistance was detected in three isolates (0.8%). TMP-SMX: Trimethoprim-sulfamethoxazole, MRSA: Methicillin-resistant Staphylococcus aureus, MSSA: Methicillin-susceptible Staphylococcus aureus

Supplementary Table S2. Multiple antibiotic resistance (MAR) index and multidrug resistance according to methicillin resistance status and clinical unit

A. By methicillin resistance status

Variable	All isolates (n=391)	MRSA (n=92)	MSSA (n=299)	p-value
MAR index, mean	0.141	0.261	0.105	<0.001a
MAR index >0.2, n (%)	99 (25.3)	52 (56.5)	47 (15.7)	<0.001b
MDR, n (%)	60 (15.3)	51 (55.4)	9 (3.0)	<0.001b
XDR or PDR, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-

B. MAR index by clinical unit

Clinical unit	n	Mean ± SD	Median (IQR)	p-value
Intensive care units	47	0.144±0.133	0.077 (0.077-0.154)	0.597c
Internal medicine units	188	0.141±0.124	0.077 (0.077-0.231)	
Paediatrics	30	0.137±0.083	0.077 (0.077-0.167)	
Emergency department	37	0.133±0.146	0.077 (0.077-0.077)	
Surgical units	89	0.144±0.124	0.077 (0.077-0.231)	
All units	391	0.141±0.124	0.077 (0.077-0.231)	

The MAR index was calculated as the number of antimicrobial agents to which an isolate was resistant divided by the number of antimicrobial agents tested: 13 agents (the 12 agents in Table 2 of the main manuscript plus penicillin) for 345 isolates and 12 agents for the 46 isolates in which penicillin was not tested; oxacillin was excluded. MDR, XDR and PDR were classified on the basis of antimicrobial categories. a: Mann-Whitney U test; b: Pearson's chi-squared test with Yates' continuity correction. c: Kruskal-Wallis H test (H=2.771). MAR: Multiple antibiotic resistance, MDR: Multidrug-resistant, XDR: Extensively drug-resistant, PDR: Pandrug-resistant, SD: Standard deviation, IQR: Interquartile range, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*

Supplementary Table S3. Pairwise co-resistance statistics for MRSA and MSSA isolates

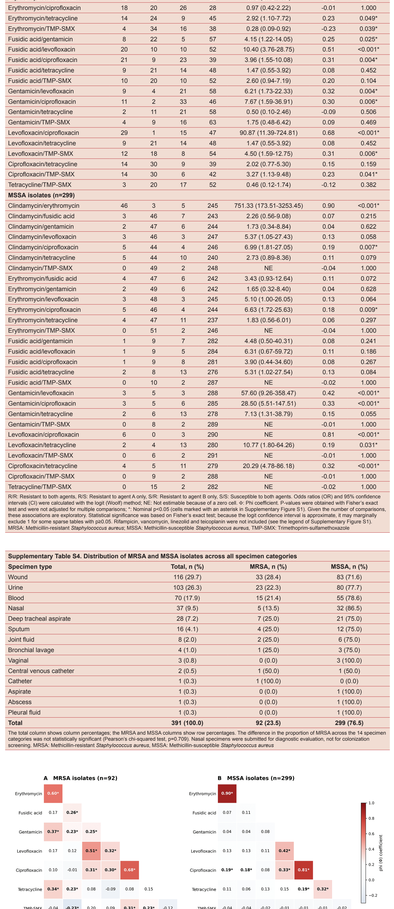
Agent A/Agent B	R/R	R/S	S/R	S/S	OR (95% CI)	Φ	p-value
MRSA isolates (n=92)							
Clindamycin/erythromycin	20	1	18	53	58.89 (7.37-470.60)	0.60	<0.001*
Clindamycin/fusidic acid	10	11	20	51	2.32 (0.85-6.30)	0.17	0.115
Clindamycin/gentamicin	8	13	5	66	8.12 (2.29-28.80)	0.37	0.001*
Clindamycin/levofloxacin	10	11	20	51	2.32 (0.85-6.30)	0.17	0.115
Clindamycin/ciprofloxacin	12	9	32	39	1.62 (0.61-4.34)	0.10	0.456
Clindamycin/tetracycline	11	10	12	59	5.41 (1.85-15.87)	0.34	0.003*
Clindamycin/TMP-SMX	4	17	16	55	0.81 (0.24-2.75)	-0.04	1.000
Erythromycin/fusidic acid	18	20	12	42	3.15 (1.28-7.78)	0.26	0.014*
Erythromycin/gentamicin	9	29	4	50	3.88 (1.10-13.72)	0.23	0.036*
Erythromycin/levofloxacin	15	23	15	39	1.70 (0.74-3.90)	0.12	0.265
Erythromycin/ciprofloxacin	18	20	26	28	0.97 (0.42-2.22)	-0.01	1.000
Erythromycin/tetracycline	14	24	9	45	2.92 (1.10-7.72)	0.23	0.049*
Erythromycin/TMP-SMX	4	34	16	38	0.28 (0.09-0.92)	-0.23	0.039*
Fusidic acid/gentamicin	8	22	5	57	4.15 (1.24-14.05)	0.25	0.025*
Fusidic acid/levofloxacin	20	10	10	52	10.40 (3.76-28.75)	0.51	<0.001*
Fusidic acid/ciprofloxacin	21	9	23	39	3.96 (1.55-10.08)	0.31	0.004*
Fusidic acid/tetracycline	9	21	14	48	1.47 (0.55-3.92)	0.08	0.452
Fusidic acid/TMP-SMX	5	20	10	52	2.60 (0.83-8.14)	0.20	0.104
Gentamicin/levofloxacin	9	4	21	58	6.21 (1.73-22.33)	0.32	0.004*
Gentamicin/ciprofloxacin	11	2	33	46	7.67 (1.59-36.91)	0.30	0.006*
Gentamicin/tetracycline	2	11	21	58	0.50 (0.10-2.46)	-0.09	0.506
Gentamicin/TMP-SMX	4	9	16	63	1.75 (0.48-6.42)	0.09	0.469
Levofloxacin/ciprofloxacin	29	1	15	47	90.87 (11.39-724.81)	0.68	<0.001*
Levofloxacin/tetracycline	9	21	14	48	1.47 (0.55-3.92)	0.08	0.452
Levofloxacin/TMP-SMX	12	18	8	54	4.50 (1.59-12.75)	0.31	0.006*
Ciprofloxacin/tetracycline	14	30	9	39	2.02 (0.77-5.30)	0.15	0.159
Ciprofloxacin/TMP-SMX	14	30	6	42	3.27 (1.13-9.48)	0.23	0.041*
Tetracycline/TMP-SMX	3	20	17	52	0.46 (0.12-1.74)	-0.12	0.382
MSSA isolates (n=299)							
Clindamycin/erythromycin	46	3	5	245	751.33 (173.51-3253.45)	0.90	<0.001*
Clindamycin/fusidic acid	3	46	7	243	2.26 (0.56-9.08)	0.07	0.215
Clindamycin/gentamicin	2	47	6	244	1.73 (0.34-8.84)	0.04	0.622
Clindamycin/levofloxacin	3	46	8	242	5.37 (1.05-27.47)	0.13	0.058
Clindamycin/ciprofloxacin	5	44	4	246	6.99 (1.81-27.05)	0.19	0.007*
Clindamycin/tetracycline	5	44	10	240	2.73 (0.89-8.36)	0.11	0.079
Clindamycin/TMP-SMX	0	49	2	248	NE	-0.04	1.000
Erythromycin/fusidic acid	4	47	2	242	3.43 (0.63-32.64)	0.11	0.072
Erythromycin/gentamicin	2	49	6	242	1.65 (0.32-8.40)	0.04	0.628
Erythromycin/levofloxacin	3	48	3	245	5.10 (1.00-26.05)	0.13	0.064
Erythromycin/ciprofloxacin	5	46	4	244	6.63 (1.72-25.63)	0.18	0.009*
Erythromycin/tetracycline	4	47	11	237	1.83 (0.56-6.01)	0.06	0.297
Erythromycin/TMP-SMX	0	51	2	246	NE	-0.04	1.000
Fusidic acid/gentamicin	1	9	7	282	4.48 (0.50-40.31)	0.08	0.241
Fusidic acid/levofloxacin	1	9	5	284	6.31 (0.67-59.72)	0.11	0.186
Fusidic acid/ciprofloxacin	1	9	8	281	3.90 (0.44-34.60)	0.08	0.287
Fusidic acid/tetracycline	2	8	13	276	5.31 (1.02-27.54)	0.13	0.084
Fusidic acid/TMP-SMX	0	10	2	287	NE	-0.02	1.000
Gentamicin/levofloxacin	3	5	3	288	57.60 (9.26-358.47)	0.42	<0.001*
Gentamicin/ciprofloxacin	3	5	6	285	28.50 (5.61-144.75)	0.33	<0.001*
Gentamicin/tetracycline	2	6	13	278	7.13 (1.31-38.79)	0.15	0.055
Gentamicin/TMP-SMX	0	8	2	289	NE	-0.01	1.000
Levofloxacin/ciprofloxacin	6	2	3	290	NE	0.81	<0.001*
Levofloxacin/tetracycline	2	4	13	280	10.77 (1.80-64.26)	0.19	0.031*
Levofloxacin/TMP-SMX	0	6	2	291	NE	-0.01	1.000
Ciprofloxacin/tetracycline	4	5	11	279	20.29 (4.78-86.18)	0.32	<0.001*
Ciprofloxacin/TMP-SMX	0	9	2	288	NE	-0.01	1.000
Tetracycline/TMP-SMX	0	15	2	282	NE	-0.02	1.000
Total	**391 (100.0)**				**92 (23.5)**	**299 (76.5)**	

The total column shows column percentages; the MRSA and MSSA columns show row percentages. The difference in the proportion of MRSA across the 14 specimen categories was not statistically significant (Pearson's chi-squared test, p=0.706). Nasal specimens were submitted for diagnostic evaluation, not for colonization screening. MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*

Supplementary Table S4. Distribution of MRSA and MSSA isolates across all specimen categories

Specimen type	Total, n (%)	MRSA, n (%)	MSSA, n (%)
Wound	116 (29.7)	33 (28.4)	83 (71.6)
Urine	109 (28.3)	23 (22.3)	80 (77.7)
Blood	70 (17.9)	15 (21.4)	55 (78.6)
Nasal	37 (9.5)	5 (13.5)	32 (86.5)
Deep tracheal aspirate	28 (7.2)	7 (25.0)	21 (75.0)
Sputum	16 (4.1)	4 (25.0)	12 (75.0)
Joint fluid	8 (2.0)	2 (25.0)	6 (75.0)
Bronchial lavage	4 (1.0)	1 (25.0)	3 (75.0)
Vaginal	3 (0.8)	0 (0.0)	3 (100.0)
Central venous catheter	2 (0.5)	1 (50.0)	1 (50.0)
Catheter	1 (0.3)	1 (100.0)	0 (0.0)
Aspirate	1 (0.3)	0 (0.0)	1 (100.0)
Abscess	1 (0.3)	0 (0.0)	1 (100.0)
Pleural fluid	1 (0.3)	0 (0.0)	1 (100.0)
Total	**391 (100.0)**	**92 (23.5)**	**299 (76.5)**

The total column shows column percentages; the MRSA and MSSA columns show row percentages. The difference in the proportion of MRSA across the 14 specimen categories was not statistically significant (Pearson's chi-squared test, p=0.706). Nasal specimens were submitted for diagnostic evaluation, not for colonization screening. MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*



* Nominal p<0.05 (Fisher's exact test), not adjusted for multiple comparisons; exploratory analysis.
Only the lower triangle is shown because the matrix is symmetric (MRSA/B = MSSA/A).

Supplementary Figure S1. Co-resistance heatmaps based on phi (Φ) coefficients. (A) MRSA isolates (n=92); (B) MSSA isolates (n=299). The eight antimicrobial agents retained for this exploratory analysis are shown. Rifampicin, vancomycin, and linezolid are not shown because no resistant isolates were identified and the phi coefficient is therefore undefined; teicoplanin is not shown because only three isolates were resistant. Cells marked with an asterisk indicate a nominally significant association (Fisher's exact test, p<0.05) without adjustment for multiple comparisons (28 agent pairs within each group); these associations should be regarded as exploratory. Because the phi coefficient is symmetric, only the lower triangle of each matrix is shown. Odds ratios, 95% confidence intervals and p-values for all pairs are given in Supplementary Table S3. MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*, TMP-SMX: Trimethoprim-sulfamethoxazole