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Cefoxitin-Susceptible mecA-Positive Staphylococcus aureus Among Pediatric Nasal Carriers: Implications for Phenotypic MRSA Detection

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Abstract

Background: Isolates that carry *mecA* but appear susceptible on phenotypic testing — described in the literature as oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) — may escape detection and lead to underestimation of methicillin-resistant *S. aureus* (MRSA) carriage. We investigated pediatric nasal carriage, associated risk factors, antimicrobial susceptibility, and cefoxitin disk-diffusion performance.

Methods: In this cross-sectional study, nasal swabs were obtained from 509 children aged 1–16 years attending pediatric outpatient clinics in Istanbul, Türkiye. *S. aureus* was identified conventionally. Methicillin resistance was assessed by cefoxitin disk diffusion according to CLSI 2018 criteria and a commercial kit-based *mecA* assay in all isolates. Risk factors were evaluated by multivariable logistic regression; diagnostic performance was assessed by sensitivity, specificity, predictive values, Cohen's κ, and Gwet's AC1.

Results: *S. aureus* carriage was 28.7% (146/508), and MRSA carriage was 2.6% (13/507). Three of the 13 *mecA*-positive isolates (23.1%) were susceptible to cefoxitin and would have been classified as methicillin-susceptible by phenotypic testing alone; this proportion rests on a denominator of only 13 isolates. Increasing age was independently associated with *S. aureus* carriage (adjusted odds ratio [OR] 1.11; 95% confidence interval [CI] 1.05–1.18) and chronic disease with MRSA carriage (adjusted OR 3.32; 95% CI 1.08–9.89). Cefoxitin disk diffusion demonstrated 76.9% sensitivity and 100% specificity. All isolates were susceptible to vancomycin, teicoplanin, linezolid, and rifampicin.

Conclusions: Phenotypic testing alone may underestimate pediatric MRSA carriage because some *mecA*-positive isolates are susceptible to cefoxitin. Molecular confirmation may be considered where accurate classification is particularly important. The diagnostic strategy may influence epidemiological estimates and should be considered when interpreting surveillance data.

Introduction

Staphylococcus aureus is a leading bacterial pathogen in children and causes diseases ranging from superficial skin and soft-tissue infections to bacteremia, osteomyelitis, pneumonia, and infective endocarditis. The anterior nares are its principal ecological niche, and asymptomatic nasal colonization is an established risk factor for subsequent infection. Invasive isolates frequently originate from strains previously colonizing the same individual, highlighting nasal carriage as a reservoir for endogenous infection and community transmission [1-5]. Nasal colonization with MRSA is therefore not merely a marker of bacterial carriage; it may also serve as a source of future infection and transmission within households and the community. Monitoring MRSA colonization and identifying associated factors in otherwise healthy children are important for characterizing community reservoirs and informing infection-prevention strategies.

The emergence of methicillin-resistant *S. aureus* (MRSA) has increased the clinical importance of accurate identification of colonized individuals. Methicillin resistance is primarily mediated by the *mecA* gene, located on the staphylococcal cassette chromosome mec, which encodes the altered penicillin-binding protein PBP2a and confers resistance to virtually all β-lactam antibiotics, the anti-MRSA cephalosporins ceftaroline and ceftobiprole being the principal exceptions. Once predominantly confined to healthcare settings, MRSA is now an important community-associated pathogen, with otherwise healthy children serving as a recognized reservoir for transmission. A recent systematic review estimated the global prevalence of pediatric MRSA colonization at approximately 5%, with considerable geographical variation according to study population, healthcare exposure, and diagnostic methodology [6-8].

An emerging diagnostic challenge is oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA). These isolates harbor the genetic determinant of methicillin resistance but may test susceptible to oxacillin and occasionally cefoxitin, resulting in misclassification as methicillin-susceptible *S. aureus* (MSSA). This pattern should be distinguished from other forms of genotype–phenotype discordance. Borderline oxacillin-resistant *S. aureus* and isolates with modified penicillin-binding proteins show reduced β-lactam susceptibility without carrying *mecA*, while heteroresistant MRSA carries *mecA* but expresses resistance in only a small subpopulation that routine testing may fail to detect. OS-MRSA differs from both in that *mecA* is present and the isolate nonetheless tests fully susceptible. Within this group, isolates that are susceptible to cefoxitin as well as to oxacillin are the most likely to escape detection altogether, because cefoxitin is the agent on which routine screening depends. Experimental and clinical evidence indicates that β-lactam exposure may select resistant subpopulations, potentially leading to microbiological persistence or treatment failure [9-16]. Exclusive reliance on phenotypic susceptibility testing may therefore underestimate MRSA carriage and influence epidemiological surveillance. Despite increasing recognition of OS-MRSA, most studies have focused on invasive isolates or hospitalized patients, whereas data from asymptomatic pediatric carriers remain scarce.

Although several studies have investigated pediatric nasal carriage of *S. aureus* in Türkiye, data incorporating systematic molecular confirmation of methicillin resistance remain scarce [7,8,17-20]. The novelty of this study lies in systematic *mecA* testing of all *S. aureus* isolates from pediatric outpatients, irrespective of phenotypic susceptibility, enabling detection of *mecA*-positive isolates that phenotypic testing would have missed and providing a more comprehensive estimate of methicillin resistance in this understudied population. We therefore aimed to determine the prevalence of nasal carriage of *S. aureus* and MRSA, including *mecA*-positive isolates that remained susceptible to cefoxitin, among pediatric outpatients; identify associated factors; characterize antimicrobial susceptibility; and assess the diagnostic performance of cefoxitin disk diffusion using molecular *mecA* detection as the reference standard.

Methods

Study design and participants

This cross-sectional study was conducted between October 2019 and January 2020, at the pediatric outpatient clinics and emergency department of Maltepe University Faculty of Medicine Hospital, Istanbul, Türkiye. Children aged 1–16 years attending during the study period were consecutively invited to participate. Children receiving intravenous chemotherapy, undergoing hemodialysis, or receiving continuous ambulatory peritoneal dialysis were excluded. Demographic and clinical data were obtained from parents or legal guardians using a standardized questionnaire. Recorded variables included age, sex, household characteristics, parental education, income, chronic disease, antibiotic use during the preceding three months, hospitalization history, healthcare utilization, and household risk factors for *S. aureus* carriage. The number of families who declined participation was not recorded. All eligible children attending during the study period were invited consecutively in order to limit selection bias, and all specimens were processed in a single laboratory according to a standardized protocol with the same quality-control strain. Children attended for routine health supervision, vaccination, or minor acute complaints; those with an active infectious illness were not excluded. Recruitment and specimen collection were completed before the COVID-19 pandemic reached Türkiye, so healthcare attendance at the study site was not affected during the sampling period. Molecular testing was carried out in July 2020, later than planned, because delivery of the PCR kits from the United Kingdom was delayed by pandemic-related disruption of international shipping; this affected the timing of the laboratory analysis only, not the composition of the cohort or the specimens themselves. No formal sample size calculation was performed; all eligible children attending during the study period were invited.

Ethics

The study was approved by the Maltepe University Clinical Research Ethics Committee on 16 October 2019 (approval no. 2019/900/61) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participants before enrollment.

Specimen collection and microbiological identification

Nasal specimens were obtained from both anterior nares using sterile cotton swabs moistened with sterile saline. Swabs were transported in Stuart medium and processed on the day of collection. Specimens were inoculated onto 5% sheep blood agar and mannitol salt agar and incubated at 35 °C for 24 hours. *S. aureus* was identified using conventional microbiological methods, including colony morphology, Gram staining, catalase testing, tube coagulase testing, and mannitol fermentation. *S. aureus* ATCC 25923 was used for quality control.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by Kirby–Bauer disk diffusion on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute M100 criteria [21]. The 28th edition

(2018) was applied because it was the version in routine use in the study laboratory when testing was performed; the breakpoints for the agents reported here, including the 30-µg cefoxitin disk criterion for *S. aureus*, are unchanged in the 29th (2019) and 30th (2020) editions. Methicillin resistance was screened using a 30-µg cefoxitin disk; inhibition-zone diameters ≤21 mm were classified as resistant. The tested agents were penicillin, cefoxitin, cefazolin, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim–sulfamethoxazole, gentamicin, rifampicin, linezolid, teicoplanin, vancomycin, fusidic acid, and chloramphenicol. Inducible clindamycin resistance was evaluated by the D-test in isolates that were resistant to erythromycin and susceptible to clindamycin. All isolates were additionally tested with the VITEK 2 automated system (bioMérieux, Marcy-l'Étoile, France) using the AST-P card, and results were compared with those obtained by disk diffusion. Because disk diffusion does not reliably detect reduced glycopeptide susceptibility in *S. aureus*, susceptibility to vancomycin and teicoplanin was determined from the minimum inhibitory concentrations generated by the automated system. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories [22]. In line with CLSI reporting rules and with the concept of inferred resistance, *mecA*-positive isolates were counted as resistant to the penicillin and cephem categories irrespective of their *in vitro* result, because detection of *mecA* implies resistance to all β-lactams other than the anti-MRSA cephalosporins. Disk-diffusion results for individual agents are nevertheless presented as measured, so that the phenotype of the discordant isolates remains visible.

mecA detection and isolate classification

All confirmed *S. aureus* isolates were evaluated for *mecA* using the commercial Primerdesign Ltd genesig Advanced Kit on a Rotor-Gene Q instrument (QIAGEN, Hilden, Germany), strictly according to the manufacturer's instructions. Testing was carried out in the laboratory of the study hospital on an instrument leased from the supplier for this purpose, by the first author together with a molecular geneticist. The assay amplifies two targets in the same reaction: *mecA*, which encodes PBP2a, and *femB*, a *S. aureus*-specific chromosomal gene included to confirm the identity of the organism. Testing was performed on DNA extracted from purified colonies grown from single-colony subcultures, not directly on clinical specimens. DNA was extracted with the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's pretreatment protocol for Gram-positive bacteria: colonies were resuspended in enzymatic lysis buffer (20 mM Tris-HCl pH 8.0, 2 mM sodium EDTA, 1.2% Triton X-100) to which lysozyme had been added to a final concentration of 20 mg/mL immediately before use, incubated to digest the cell wall, and then processed by the standard column-based purification procedure. Each run included an internal amplification control together with positive and negative controls, and extraction and amplification were carried out in separate areas to limit carry-over contamination. An isolate was reported as *mecA*-positive when exponential amplification curves crossed the manufacturer-defined threshold within the validated cycle range for both *femB* and *mecA*; amplification of *femB* in every isolate confirmed that the organism tested was *S. aureus*. Isolates carrying *mecA* were classified as MRSA irrespective of phenotypic susceptibility. For every isolate in which the *mecA* result was discordant with the cefoxitin phenotype, the assay was repeated

independently on a separate occasion using a fresh subculture, and in each case the original result was reproduced. *mecA*-positive isolates that remained susceptible to cefoxitin by disk diffusion are referred to throughout as cefoxitin-susceptible *mecA*-positive isolates. Because oxacillin minimum inhibitory concentrations were not determined, the term oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA) is reserved for the published literature, in which oxacillin susceptibility has been demonstrated directly.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequencies and percentages. Between-group comparisons used Student's t test or one-way analysis of variance for continuous variables and Pearson's χ^2 or Fisher's exact test for categorical variables, as appropriate. Ten candidate predictors were examined in univariable logistic regression, and those associated with the outcome at $P < 0.10$ were entered into the corresponding multivariable model. Because only 13 children carried *mecA*-positive *S. aureus*, multivariable models were fitted by Firth penalized logistic regression, which reduces small-sample bias, with confidence intervals obtained from the profile penalized likelihood; unpenalized maximum-likelihood models were fitted in parallel for comparison. Crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) are reported together. Household income was not entered into the multivariable models because the value was missing for 206 of 507 participants, and was instead examined in a sensitivity analysis restricted to records with income available. Cefoxitin disk diffusion was evaluated against molecular *mecA* detection by calculating sensitivity, specificity, positive and negative predictive values, overall agreement, Cohen's κ, and Gwet's AC1. The absolute difference between phenotypically and molecularly defined MRSA carriage was estimated with a confidence interval for paired proportions, and the discordance was tested with an exact binomial (McNemar) test. Because of sparse cells, 95% CIs for κ and AC1 were estimated using 10,000 bootstrap resamples. Two-sided $P < 0.05$ was considered statistically significant. All analyses were performed on complete cases; no imputation was undertaken. Age was analysed as a continuous variable and, for descriptive purposes, in four groups (1–3, 4–6, 7–10, and 11–16 years).

Results

Study population and prevalence of nasal carriage

A total of 509 children aged 1–16 years were enrolled (Fig. 1). The culture result was missing for one participant and *mecA* testing was not performed for one *S. aureus* isolate; these records were excluded from the corresponding analyses, leaving 507 children in the comparison by carriage status, of whom 255 of 503 with recorded sex (50.7%) were male (mean age: 6.05 ± 3.52 years). *Staphylococcus aureus* was isolated from 146 of the 508 children with an available culture result, corresponding to a nasal carriage prevalence of 28.7% (95% CI: 25.0–32.8). Among the 145 isolates tested for *mecA*, 132 (91.0%)

were *mecA*-negative and 13 (9.0%) carried *mecA*, yielding an overall MRSA carriage prevalence of 2.6% (95% CI: 1.5–4.3). Demographic and clinical characteristics according to carriage status are presented in Table 1.

Table 1. Demographic and clinical characteristics by nasal carriage status (N = 507).

Characteristic	Non-carrier	MSSA	MRSA	p value
n	362	132	13	
Age, years, mean ± SD	5.62 ± 3.47	7.03 ± 3.44	7.81 ± 3.31	<0.001
Male sex, n (%)	177 (49.3)	70 (53.4)	8 (61.5)	0.526
Chronic disease, n (%)	63 (17.5)	27 (20.6)	6 (46.2)	0.031
Antibiotic use in previous 3 months	165 (46.0)	44 (33.6)	6 (46.2)	0.048

MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; SD, standard deviation. Percentages are calculated on the number of participants with the variable recorded. The culture result was missing for one participant and *mecA* testing was not performed for one *S. aureus* isolate; both were excluded from this comparison. Sex was not recorded or was invalid for four participants, and chronic disease and recent antibiotic use were not recorded for three and four participants, respectively. P values are global comparisons across the three carriage groups (one-way analysis of variance for age; Pearson's χ^2 test for categorical variables).

Age distribution and factors associated with nasal carriage

The mean ages of children without carriage, those colonized with MSSA, and those colonized with MRSA were 5.62 ± 3.47, 7.03 ± 3.44, and 7.81 ± 3.31 years, respectively. *S. aureus* carriage increased from 16.7% among children aged 1–3 years to 42.4% among those aged 11–16 years (P < 0.001; Table 2). Three of the ten candidate predictors were associated with *S. aureus* carriage at P < 0.10 in univariable analysis and entered the corresponding model. After adjustment, increasing age remained independently associated with carriage (adjusted OR 1.11; 95% CI 1.05–1.18; P < 0.001), whereas neither antibiotic use in the previous three months (adjusted OR 0.74; 95% CI 0.48–1.12) nor a healthcare-visit interval longer than one month (adjusted OR 1.67; 95% CI 0.94–3.12) retained statistical significance, although both had been associated with carriage before adjustment. For MRSA carriage, age and chronic disease met the screening criterion; chronic underlying disease was independently associated with carriage (adjusted OR 3.32; 95% CI 1.08–9.89; P = 0.036) while age was not (adjusted OR 1.13; 95% CI 0.97–1.31). Penalized and unpenalized estimates were closely similar for both outcomes. Sex, number of siblings, previous hospitalization of the child or of a household member, history of an invasive procedure, and chronic disease in a household member did not meet the screening criterion for either outcome (Table 3).

Table 2. Nasal *S. aureus* and MRSA carriage by age group.

Age, years	n	*S. aureus*, n (%; 95% CI)	MRSA, n (%; 95% CI)
1–3	138	23 (16.7; 11.4–23.8)	1 (0.7; 0.1–4.0)
4–6	165	44 (26.7; 20.5–33.9)	4 (2.4; 0.9–6.1)
7–10	138	51 (37.0; 29.4–45.3)	5 (3.6; 1.6–8.2)
11–16	66	28 (42.4; 31.2–54.4)	3 (4.5; 1.6–12.5)
Total	507	146 (28.8; 25.0–32.9)	13 (2.6; 1.5–4.3)

Trend across age groups for *S. aureus* carriage: Cochran–Armitage $\chi^2 = 20.4$, $p < 0.001$. Age was missing for one participant and the culture result for another; both were excluded from this table. The 507 children analysed here are therefore not the same 507 as in Table 1, which instead excludes the one isolate without a *mecA* result: of the 146 carriers shown here, 145 had a *mecA* result and are classified as MSSA or MRSA in Table 1. One participant recorded as younger than one year is included in the 1–3 years group. CI, confidence interval (Wilson).

Table 3. Crude and adjusted associations with nasal *S. aureus* and MRSA carriage, presented separately for each outcome.

Predictor	Crude OR (95% CI)	P	Adjusted OR (95% CI)	P
***S. aureus* carriage (n = 502; 144 carriers)**				
Age, per year	1.13 (1.07–1.19)	<0.001	1.11 (1.05–1.18)	<0.001
Antibiotic use in previous 3 months	0.63 (0.42–0.93)	0.022	0.74 (0.48–1.12)	0.149
Healthcare visit interval >1 month	2.14 (1.20–3.82)	0.010	1.67 (0.94–3.12)	0.083
MRSA carriage (n = 503; 13 carriers)				
Age, per year	1.14 (0.99–1.33)	0.073	1.13 (0.97–1.31)	0.127
Chronic disease	3.82 (1.25–11.64)	0.018	3.32 (1.08–9.89)	0.036

CI, confidence interval; OR, odds ratio. Adjusted estimates are from Firth penalized logistic regression with profile penalized-likelihood confidence intervals. Ten candidate predictors were screened in univariable analysis (age, sex, chronic disease, antibiotic use in the previous three months, healthcare visit interval, hospitalization of a household member in the previous year, hospitalization of the child in the previous year, history of an invasive procedure, number of siblings, and chronic disease in a household member); those associated with the outcome at $P < 0.10$ entered the corresponding multivariable model. The healthcare visit interval was recorded in three categories (more than once a

month; once every one to six months; once every six to twelve months) and was dichotomised for analysis into an interval of one month or less versus longer than one month. Reference categories: healthcare visit interval ≤1 month; no antibiotic use in the previous three months; no chronic disease. Household income was not included because the value was missing for 206 of 507 participants; a sensitivity analysis restricted to participants with income recorded (n = 300) gave an adjusted OR for age of 1.15 (95% CI 1.07–1.24) and an adjusted OR for income of 0.76 (95% CI 0.54–1.07) per category, leaving the conclusions unchanged.

Antimicrobial susceptibility patterns

Penicillin resistance was most frequent (92.4%), followed by erythromycin (19.0%), clindamycin (14.5%), tetracycline (6.9%), cefoxitin (6.8%), cefazolin (2.1%), ciprofloxacin (2.1%), and trimethoprim–sulfamethoxazole (0.7%). Inducible clindamycin resistance was detected in one of the six isolates eligible for the D-test (16.7%), corresponding to 0.7% of all isolates. All isolates were susceptible to vancomycin, teicoplanin, linezolid, rifampicin, gentamicin, chloramphenicol, and fusidic acid. Applying the CLSI reporting rule for *mecA*-positive isolates, multidrug resistance was identified in 17.1% of isolates overall and was more frequent among *mecA*-positive than *mecA*-negative isolates (46.2% versus 14.4%; P = 0.011). Resistance to cefoxitin (76.9% versus 0%), cefazolin (25.0% versus 0%) and tetracycline (41.7% versus 3.8%) was more frequent among *mecA*-positive than *mecA*-negative isolates (all P < 0.001), whereas resistance to the remaining agents did not differ between the two groups (Table 4).

Table 4. Antimicrobial resistance among 146 nasal *S. aureus* isolates, overall and according to *mecA* status.

Antimicrobial agent	All isolates (n = 146)	*mecA*-negative (n = 132)	*mecA*-positive (n = 13)	p value
Penicillin	134/145 (92.4)	122/132 (92.4)	11/12 (91.7)	1.000
Cefoxitin	10/146 (6.8)	0/132 (0.0)	10/13 (76.9)	<0.001
Cefazolin	3/145 (2.1)	0/132 (0.0)	3/12 (25.0)	<0.001
Erythromycin	27/142 (19.0)	24/129 (18.6)	3/12 (25.0)	0.700
Clindamycin	21/145 (14.5)	19/132 (14.4)	2/12 (16.7)	0.688
Inducible clindamycin resistance (D-test positive)	1/6 (16.7)	1/5 (20.0)	0/1 (0.0)	–
Tetracycline	10/145 (6.9)	5/132 (3.8)	5/12 (41.7)	<0.001
Ciprofloxacin	3/145 (2.1)	3/132 (2.3)	0/12 (0.0)	1.000
Trimethoprim–sulfamethoxazole	1/145 (0.7)	0/132 (0.0)	1/12 (8.3)	0.083
Gentamicin, rifampicin, fusidic acid, chloramphenicol, linezolid, teicoplanin, vancomycin	0/145 (0.0)	0/132 (0.0)	0/12 (0.0)	–
Multidrug resistance	25/146 (17.1)	19/132 (14.4)	6/13 (46.2)	0.011

Data are n resistant / n tested (%). Denominators differ between agents because susceptibility results were unavailable for one to four isolates, and because *mecA* was not performed on one *S. aureus* isolate, which is therefore counted in the overall column only. p values compare *mecA*-negative with *mecA*-positive isolates (Fisher's exact test); they are not calculated where no isolate in either group was resistant or where the denominator was below five. The D-test was performed only in the six isolates that were resistant to erythromycin and susceptible to clindamycin, so inducible clindamycin resistance corresponds to 1 of 145 isolates (0.7%) overall. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, with *mecA*-positive isolates counted as resistant to the penicillin and cephem categories irrespective of their *in vitro* result. The cefazolin and cefoxitin rows show measured disk-diffusion results; under CLSI reporting rules a *mecA*-positive isolate is reported clinically as resistant to cefazolin and to the other conventional β-lactams whatever the disk result, so the nine *mecA*-positive isolates that appeared cefazolin-susceptible *in vitro* would be reported as resistant. CI, confidence interval (Wilson).

Diagnostic performance of cefoxitin disk diffusion

Using molecular *mecA* detection as the reference standard, cefoxitin disk diffusion demonstrated 76.9% sensitivity and 100% specificity, with positive and negative predictive values of 100% and 97.8%; overall

agreement was 97.9%, Cohen's κ 0.859 and Gwet's AC1 0.976. Confidence intervals for all of these estimates are given in Table 5 and are wide for sensitivity, reflecting the small number of *mecA*-positive isolates. Cefoxitin correctly identified 10 of 13 *mecA*-positive isolates; the remaining three were phenotypically susceptible and susceptible to cefoxitin, corresponding to 3 of the 13 *mecA*-positive isolates (23.1%) and to 2.1% of all *S. aureus* isolates (Table 5). Expressed at the level of the cohort, phenotypic testing identified MRSA carriage in 10 of 507 children (2.0%) compared with 13 of 507 (2.6%) by *mecA* detection, an absolute difference of 0.59 percentage points (95% CI −0.08 to 1.26; exact McNemar P = 0.25). The three discordant isolates were recovered from children aged 11, 9 and 7 years; one had a chronic underlying condition and one had received antibiotics in the preceding three months. All three were resistant to penicillin and one additionally to erythromycin, and all three were susceptible to every other agent tested (Table 6).

Table 5. Agreement between cefoxitin disk diffusion and *mecA* PCR, and the resulting diagnostic performance, among the 145 *S. aureus* isolates tested for *mecA*.

Cefoxitin disk diffusion result	***mecA*-positive**	***mecA*-negative**	**Total**
Resistant	10	0	10
Susceptible	3*	132	135
Total	13	132	145
Diagnostic performance	**Estimate**	**95% CI**	
Sensitivity	76.9%	49.7–91.8	
Specificity	100.0%	97.2–100.0	
Positive predictive value	100.0%	72.2–100.0	
Negative predictive value	97.8%	93.7–99.2	
Overall agreement	97.9%	94.1–99.3	
Cohen's κ	0.859	0.651–1.000	
Gwet's AC1	0.976	0.943–1.000	

*These three isolates carried *mecA* but were susceptible to cefoxitin by disk diffusion, corresponding to 23.1% of the 13 *mecA*-positive isolates and to 2.1% of all *S. aureus* isolates; their characteristics are given in Table 6. Oxacillin minimum inhibitory concentrations were not determined. Estimates of diagnostic performance are based on 13 *mecA*-positive and 132 *mecA*-negative isolates, and the confidence interval for sensitivity is correspondingly wide. Confidence intervals for κ and AC1 were estimated with 10,000 bootstrap resamples. CI, confidence interval.

Table 6. Characteristics of the three children carrying cefoxitin-susceptible *mecA*-positive *S. aureus* and the resistance profile of their isolates.

Isolate	Age (years)	Sex	Chronic disease	Antibiotic use in previous 3 months	Resistant to
1	11	Female	No	No	Penicillin
2	9	Male	Yes (asthma)	No	Penicillin, erythromycin
3	7	Male	No	Yes	Penicillin

All three isolates carried *mecA* and amplified *femB*, confirming the species, and the *mecA* result was reproduced on independent repeat testing from a fresh subculture in each case. All three were susceptible by disk diffusion to cefoxitin and to every other agent tested apart from those listed, namely cefazolin, clindamycin, tetracycline, ciprofloxacin, trimethoprim–sulfamethoxazole, gentamicin, rifampicin, fusidic acid, chloramphenicol and linezolid, and to teicoplanin and vancomycin by automated MIC determination; none was D-test positive. Under CLSI reporting rules each would be reported clinically as resistant to all β-lactams other than the anti-MRSA cephalosporins. Oxacillin minimum inhibitory concentrations and PBP2a testing were not performed, and the isolates are no longer available.

Discussion

The most important finding of the present study is that reliance on routine phenotypic susceptibility testing alone may underestimate methicillin resistance among pediatric nasal *Staphylococcus aureus* carriers because some *mecA*-positive isolates are susceptible to cefoxitin. Cefoxitin disk diffusion agreed closely with molecular *mecA* detection overall, and no *mecA*-negative isolate was misclassified as resistant; the point estimate for specificity was 100%, although with 132 *mecA*-negative isolates this cannot be read as evidence of perfect specificity. Sensitivity, by contrast, was 76.9%: nearly one-quarter of the genetically confirmed MRSA isolates were susceptible to cefoxitin and would have been reported as MSSA without molecular testing. The diagnostic approach itself may therefore influence epidemiological estimates of MRSA colonization. Recent studies similarly show that conventional susceptibility testing can fail to identify *mecA*-positive isolates, potentially underestimating MRSA prevalence and complicating therapeutic decisions [9-16].

Although *S. aureus* and MRSA carriage in our cohort was comparable with previous pediatric reports from Türkiye and other countries, the principal contribution of this study lies in systematic molecular confirmation rather than the prevalence estimate. For context, the nationwide Spanish COSACO study reported *S. aureus* and MRSA carriage prevalences of 33.0% and 1.44%, respectively, while recent global analyses have emphasized substantial between-study heterogeneity [7,8]. A 2024 systematic review and meta-analysis of more than 100 pediatric studies estimated pooled global MRSA colonization at approximately 5% and demonstrated substantial variation according to geography, healthcare exposure, demographic characteristics, and laboratory methodology [6-8]. Unlike most pediatric carriage studies, all *S. aureus* isolates in our cohort underwent *mecA* assessment irrespective of phenotypic susceptibility. This strategy identified cefoxitin-susceptible *mecA*-positive isolates that would otherwise have remained undetected and provided a more complete estimate of methicillin resistance.

Recognition of OS-MRSA challenges the assumption that phenotypic susceptibility reliably excludes methicillin resistance. Genomic and experimental studies indicate that heterogeneous *mecA* expression, changes in regulatory pathways, and variable PBP2a production contribute to this phenotype [9-11,13-15]. From a diagnostic perspective, such phenotype-genotype discordance may result in misclassification of OS-MRSA as methicillin-susceptible *S. aureus* when routine susceptibility testing is used alone [23]. This may influence antimicrobial selection and lead to underestimation of the true MRSA carriage burden in epidemiological surveillance. Apparently susceptible isolates may rapidly express high-level beta-lactam resistance following antimicrobial exposure, meaning that phenotypic susceptibility does not necessarily reflect their biological resistance potential [9,10,13,14,16]. Clinical reports of invasive infection further illustrate the potential treatment implications [12,14,16]. Whereas previous reports have predominantly involved invasive isolates or hospitalized patients, the identification of cefoxitin-susceptible *mecA*-positive isolates among asymptomatic pediatric carriers raises the possibility that this phenotype circulates in the community before clinically apparent infection develops.

Where molecular confirmation earns its place is determined less by how often the discordant phenotype occurs than by the asymmetry of the consequences of misclassifying it. In carriage surveillance, where a misclassified isolate shifts an estimate but not a treatment decision, cefoxitin disk diffusion remains a reasonable screening method. The calculus changes when the organism is driving disease and a β-lactam is to be relied upon as definitive therapy: in staphylococcal bacteraemia, endocarditis, or osteoarticular and other deep-seated infection, a falsely susceptible result commits the patient to a regimen under which a resistant subpopulation may be selected, an outcome documented in both experimental work and clinical reports [12,14,16]. The same reasoning applies when microbiological persistence or clinical failure follows apparently appropriate β-lactam therapy, and when a carriage result will govern decolonisation or surgical prophylaxis in an immunocompromised child or one awaiting implant surgery. Outbreak investigation forms a separate case, in which the accuracy of classification determines whether transmission is recognised at all.

Increasing age was independently associated with *S. aureus* carriage, whereas chronic underlying disease was the only factor independently associated with MRSA colonization. These findings are biologically plausible and broadly consistent with evidence identifying healthcare exposure, underlying medical conditions, and previous antibiotic use as determinants of pediatric MRSA colonization [6-8,19,20,24,25]. The antimicrobial susceptibility profile was reassuring: all isolates were susceptible to vancomycin, teicoplanin, linezolid, rifampicin, gentamicin, chloramphenicol, and fusidic acid, while resistance to other non-β-lactam agents was relatively uncommon. Multidrug resistance was nevertheless more frequent among MRSA than MSSA isolates. Recent antibiotic use was, in contrast, less frequent among carriers than among non-carriers in univariable analysis. This association did not persist after adjustment for age and frequency of healthcare contact, which points to confounding rather than to a direct effect: children who attend more often are both more likely to have received antibiotics and differ in age distribution from those who attend rarely. Transient suppression of colonization after β-lactam exposure remains a possible contributor, but the cross-sectional design cannot establish the

temporal sequence of exposure and colonization, and this observation is best regarded as hypothesis-generating.

A key strength of this study was the systematic evaluation of the *mecA* gene in all *Staphylococcus aureus* isolates, regardless of phenotypic susceptibility. This approach revealed the cefoxitin-susceptible *mecA*-positive isolates that phenotypic testing alone would have missed. Additional strengths include prospective enrollment of a relatively large pediatric outpatient cohort and simultaneous assessment of prevalence, associated factors, antimicrobial susceptibility, and diagnostic performance. Several limitations should be acknowledged. The single-center design and limited number of MRSA isolates reduced the precision of MRSA-specific analyses. Only 13 children carried *mecA*-positive *S. aureus*, so the multivariable model for MRSA carriage rested on 6.5 outcome events per candidate predictor, below the figure conventionally regarded as adequate. Firth penalized regression was used for this reason and produced estimates closely similar to the unpenalized model, which argues against gross sparse-data bias; nevertheless the association between chronic underlying disease and MRSA carriage is wide in its confidence interval, should be regarded as exploratory rather than as an established independent predictor, and requires confirmation in larger cohorts. Household income was missing for 206 of the 507 participants in the analysis set, because the socioeconomic section of the questionnaire was not transcribed for part of the cohort, and parental education was missing for almost the same participants. These variables were therefore kept out of the main models and examined only in a sensitivity analysis. Participants with and without income recorded did not differ in age or in *S. aureus* carriage, which argues against informative missingness, but residual confounding by socioeconomic position cannot be excluded. Only the anterior nares were sampled. The isolates were stored until molecular testing was completed but were not retained thereafter and are no longer available, so confirmatory investigations that would ordinarily be used to characterise genotype–phenotype discordance could not be undertaken. In particular, oxacillin minimum inhibitory concentrations were not determined, PBP2a expression was not assessed by an immunological assay, and *mecA* positivity could not be reconfirmed with an assay targeting a different region of the gene; *mecC* was likewise not sought. Inhibition zones were recorded as the categorical interpretation rather than as the measured diameter, so the zone sizes of the discordant isolates cannot be reported, and it is not possible to establish retrospectively whether any of them fell close to the breakpoint. The discordant isolates are therefore defined by cefoxitin susceptibility rather than by demonstrated oxacillin susceptibility, and although the *mecA* result was independently reproduced for each of them, an alternative explanation for the discordance cannot be formally excluded. Molecular characterization beyond *mecA*—including SCCmec, spa and multilocus sequence typing, virulence-gene profiling, and whole-genome sequencing—was also not performed. These methods would provide further insight into the clonal relationships and transmission dynamics of OS-MRSA. Finally, the specimens were collected in 2019–2020 and susceptibility results were interpreted according to the CLSI M100 criteria then in use in the study laboratory (28th edition, 2018). The findings therefore describe pediatric nasal carriage before the COVID-19 pandemic and before the subsequent shifts in antibiotic use and healthcare contact that accompanied it; contemporary carriage patterns may differ, and the estimates reported here are best read as a pre-pandemic baseline. Because the number of

families who declined participation was not recorded, the participation rate cannot be calculated and the extent to which the cohort represents the wider pediatric population of the catchment area is uncertain; children attending hospital-based services may differ from children in the community, and the estimates reported here should not be extrapolated to healthy children generally without this qualification.

Conclusions

In this pre-pandemic cohort of pediatric outpatients, 3 of 13 *mecA*-positive *S. aureus* isolates were susceptible to cefoxitin and would have been reported as methicillin-susceptible had phenotypic testing been used alone. These findings show that genotype–phenotype discordance occurs among asymptomatic pediatric carriers. They do not, on the basis of three isolates from a single centre and without independent microbiological confirmation, establish how often the phenotype occurs, whether it carries clinical consequences, or that routine molecular confirmation is warranted in surveillance. Larger multicentre studies with confirmatory susceptibility testing and molecular typing are needed to answer those questions.

Abbreviations

AC1, agreement coefficient 1; aOR, adjusted odds ratio; ATCC, American Type Culture Collection; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; MDR, multidrug resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; OS-MRSA, oxacillin-susceptible *mecA*-positive methicillin-resistant *Staphylococcus aureus*; PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; SD, standard deviation.

Declarations

Ethics approval and consent to participate

The study was approved by the Maltepe University Clinical Research Ethics Committee on 16 October 2019 (approval no. 2019/900/61) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participants before enrollment.

Consent for publication

Not applicable.

Availability of data and materials

The de-identified data supporting the findings of this study are available from the corresponding author on reasonable request and subject to applicable institutional and ethical requirements.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

APK contributed to the investigation, data collection, laboratory work, data analysis, and drafting of the manuscript. IAT contributed to the study conception, supervision, methodology, and critical revision of the manuscript. MES contributed to the interpretation of laboratory data. AK contributed to the interpretation of microbiological data. UD contributed to the microbiological assessment and interpretation of microbiological data. All authors critically reviewed the manuscript, read and approved the final version, and agree to be accountable for all aspects of the work.

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Figures

Fig. 1 Participant flow

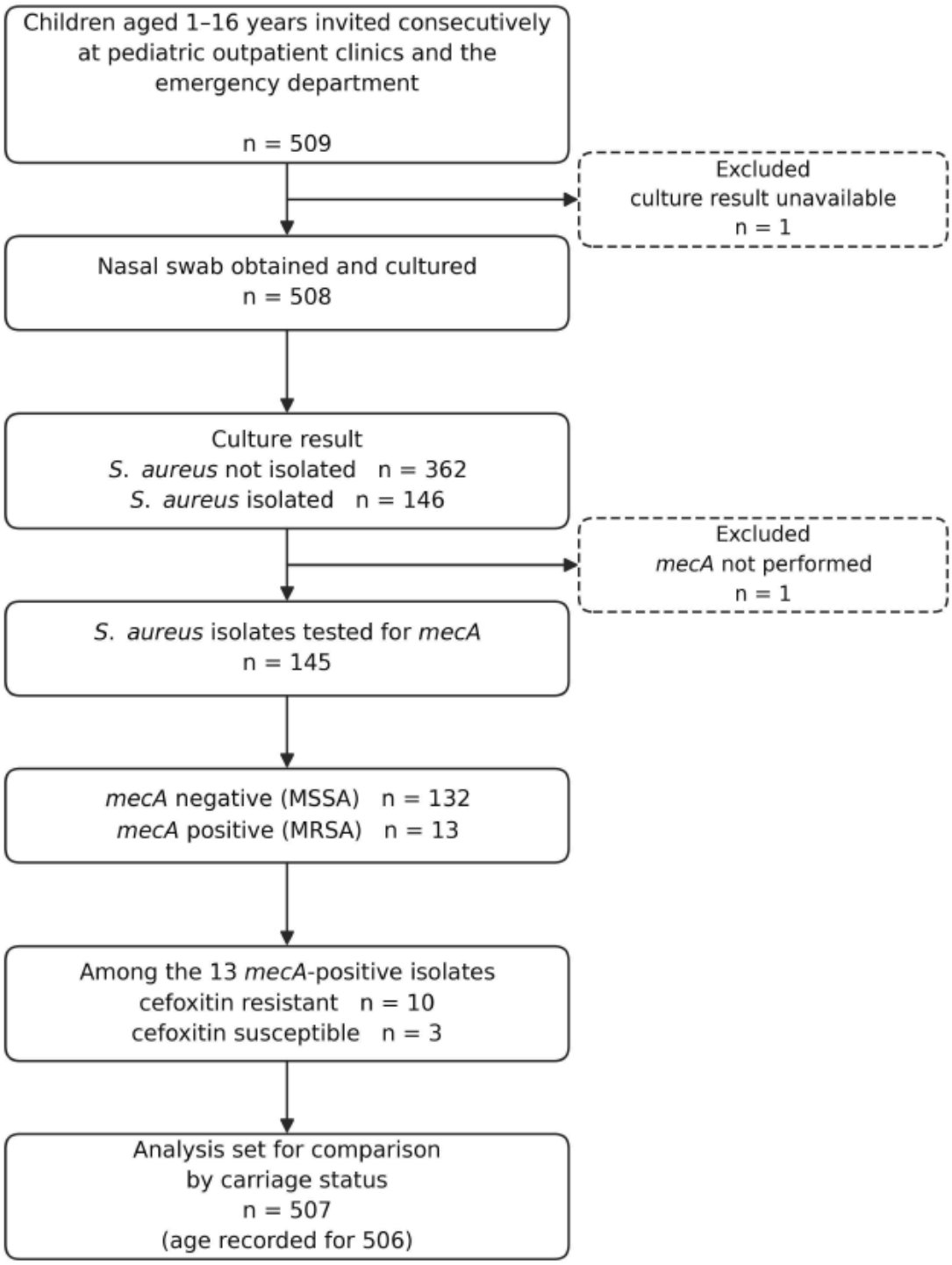


Figure 1

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