RESEARCH PAPER

Antibiotic Susceptibility, Carrier State and Predictors of Outcome of *Staphylococcus aureus* Infections in Hospitalized Children

KIRANPREET KAUR,[1] SUMAIRA KHALIL,[1] NP SINGH,[2] POOJA DEWAN,[1] PIYUSH GUPTA,[1] DHEERAJ SHAH[1]

Departments of [1]Pediatrics and [2]Microbiology, University College of Medical Sciences (University of Delhi) and GTB Hospital, Delhi.

Correspondence to:
Dr Dheeraj Shah, Director-Professor, Department of Pediatrics, University College of Medical Sciences & GTB Hospital, Delhi 110095.
dshah@ucms.ac.in
Received: Sept 05, 2022;
Initial review: Sept 28, 2022;
Accepted: Nov 14, 2022.

Objectives: To evaluate the antibiotic resistance pattern, clinical profile and predictors for adverse outcomes in children hospitalized due to staphylococcal infection; and the frequency of nasal and axillary carrier states in these children. **Methods**: This descriptive study enrolled 100 symptomatic children (aged 1 month - 12 years) in whom *S. aureus* was isolated from cultures of blood, pus or cerebrospinal fluid. All samples were processed as per the Clinical and Laboratory Standards Institute (CLSI) standards. Antimicrobial susceptibility was tested using disc diffusion method; minimum inhibitory concentration (MIC) for vancomycin was measured using E strips. Predictors for poor recovery were determined by univariate and multivariable logistic regression analysis. **Results**: Skin and soft tissue infections were the most common (47%) followed by respiratory infections (37%). Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 62%, out of which 63% (39/62) were multi-drug resistant. Carrier state was present in 49% (93% MRSA); 80% were axillary carriers. High MIC (>1 µg/mL) for vancomycin was seen in 65% of patients, and was the only factor associated with poor recovery [aOR (95%CI) 5.3 (1.6,18.5); *P*=0.008] on multivariable logistic regression analysis. **Conclusion:** MRSA is the predominant strain in severe staphylococcal infections requiring hospitalization, and majority of them are multidrug resistant. High MIC to vancomycin among *S. aureus* is an emerging concern.

Keywords: *Antimicrobial resistance, Methicillin-resistance, Treatment failure*.

Published online: November 19, 2022; ***PII***: S097475591600469

Staphylococcus aureus is associated with significant morbidity and mortality in children, especially in low- and middle-income countries (LMICs). Community-acquired MRSA (CA-MRSA) infections have a fundamentally different epidemiology compared to hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. In India, the overall rate of MRSA in clinical specimens is reported to be high (45-60%) [2].

Less is understood about community-acquired *S. aureus* and predictors of adverse outcomes in children. There are conflicting data on whether higher vancomycin minimum inhibitory concentrations (MICs) adversely affects outcome in patients with *S. aureus* bacteremia [3]. Moreover, there is paucity of recent data related to the profile and resistance pattern of *S. aureus* infections in children. Thus, we conducted this study to describe the antibiotic resistance pattern, clinical profile and predictors for adverse outcomes in children hospitalized due to staphylococcal infection. We also aimed to study the frequency of nasal and axillary carrier states in these children.

METHODS

This observational study was conducted in the Departments of Pediatrics and Microbiology of a medical-college-affiliated public hospital, from November, 2017 to April, 2019. We included children aged 1 month to 12 years with features of clinical sepsis such as fever, chills, deep abscesses, hypotension or oliguria, and where *S. aureus* was isolated from cultures of blood, pus or cerebrospinal fluid (CSF). Culture isolate was considered a contaminant if the patient's clinical features and laboratory test did not suggest infection, follow up blood cultures were negative when the patient did not receive any antibiotic, patient recovered without any anti-staphylococcal treatment, or there was a polymicrobial growth; such patients were excluded from the study. Informed consent was obtained from parents or guardians of every participant. Assent was obtained from children 7 years of age or older. An approval from the institutional ethics committee was obtained.

A detailed history and physical examination were recorded for all participants. Complete hemogram and

blood culture were performed on all, pus and CSF cultures were collected, wherever relevant. Other investigations (ultrasonography, chest *X*-ray, echocardiography, computed tomography (CT) scan) were guided by the clinical symptoms and response to therapy.

Peripheral venous blood (1-3 mL) was drawn by aseptic method and inoculated in BACTEC 9120 bottles for culture. Samples for blood culture were collected at admission and every 48-72 hours till clearance. All the clinical samples (blood, pus, CSF, and other body fluids) were inoculated on 5% sheep blood agar and MacConkey agar plates. After overnight incubation, isolates were identified by their colony characters, morphology and staining characters. Antimicrobial susceptibility testing (AST) was done on Muller Hilton agar (MHA) by modified Kirby Bauer disc diffusion and interpreted using Clinical and Laboratory Standards (CLSI) guidelines [4]. E-test was performed to determine the susceptibility to vancomycin and linezolid. Vancomycin MIC determination was performed by agar strip method, ATCC 29213 *Staphylococcus aureus* was used as control stain and the MIC's for the control strain were found within susceptible breakpoints. To determine the nasal carrier state, one sterile cotton swab was inserted approximately 2 cm into both nostrils and rotated against the anterior nasal mucosa for 3 seconds. Similarly, axillary swabs were obtained.

All children were managed according to the National Centre for Disease Control guidelines [5]. Empirical therapy was started with amoxicillin-clavulanic acid or cloxacillin (± gentamicin). In serious infections, vancomycin was started by the treating physician at their discretion. Antibiotics were changed as per the susceptibility pattern of the organisms. For uncomplicated infections, minimum duration of treatment was two weeks. For complicated infections, liver abscess, empyema and endocarditis, treatment duration varied from 21 to 42 days from negative blood cultures depending on their clinical response [6].

The outcome was measured in terms of antibiotic resistance pattern, type of infection, clinical recovery, and nasal and axillary carriage of *S. aureus.* Isolates resistant to methicillin (cefoxitin) were labelled as MRSA [4] and those resistant to vancomycin were labelled as Vancomycin-resistant *S. aureus* (VRSA) [4]. MRSA isolates found to be resistant to three or more other antibiotics apart from beta-lactams were labelled as multidrug resistant (MDR) *S. aureus* [7]. Recovery was defined in terms of duration of fever (from enrolment to beginning of an afebrile period of 72 hours), appetite, duration of hospitalization and microbiological clearance (negative blood culture at 48-72 hour). Poor recovery was defined by fever lasting more than 7 days, poor appetite for more than 72 hours, hospitalization for more than 14 days, delayed microbiological clearance more than 72 hours, antibiotic resistance, and complications (shock, encephalopathy, ventilatory support or death).

Using the proportion of 54% MRSA amongst total staphylococcus infections in a previous study from India [7], we calculated a sample size of 96 participants with 10% absolute precision at 95% confidence level and alpha error of 0.05. We planned to enrol 100 children with confirmed *S. aureus* infection.

Statistical analysis: The data were entered into Microsoft Excel and analyzed using SPSS 20. Descriptive statistics were performed for antibiotic resistance pattern, clinical profile, nasal and axillary carrier rate, and outcome parameters. For risk factors of poor recovery, various parameters were compared between children having poor recovery against those not having poor recovery. Student *t* test was used to compare continuous variables, and chi-square test or Fischer exact test was used to compare categorical variables. Logistic regression analysis was performed for risk factors with *P* value ≤0.25 on univariate analysis to calculate adjusted odds ratio and 95% confidence interval for the outcomes of poor recovery.

RESULTS

We enrolled 100 children (56 boys) with *S. aureus* infections with one-third (*n*=31) of them aged less than 6 months and nearly half (*n*=51) were infants. Majority of the

Table I Clinical Diagnosis of Children With Staphylococcal Infections Enrolled in the Study (*N*=100)

Diagnosis	*No. (%)*
Skin and soft tissue infections	
Abscess	30 (30)
upper limb	4 (4)
lower limb	13 (13)
both upper and lower limb	2 (2)
head and neck	6 (6)
trunk and back	5 (5)
Pyoderma/cellulitis	9 (9)
Necrotizing soft tissue infection	8 (8)
Bone and joint infections	
Septic arthritis	8 (8)
Osteomyelitis	2 (2)
Osteomyelitis and septic arthritis	2 (2)
Pneumonia	29 (29)
Pneumonia with complications[a]	8 (8)
Acute meningitis	9 (9)
Sepsis without focus	14 (14)

Total percentage exceeds 100 because of the presence of more than one diagnosis in some children. [a]empyema/pneumothorax.

patients had acute complaints with mean (SD) duration of illness of 11.1 (20.3) days. Eighty-four children presented with fever and one third (*n*=28) had associated chills and rigors. Majority of participants were undernourished with mean (SD) weight for age *z*-score (WAZ), height for age *z*-score (HAZ) and weight for height *z*-score (WFHZ) as -2.9 (1.7), -2.1 (1.7) and -2.3 (1.5), respectively. Three-fourths (77%) of the participants were anemic, and 50% had leukocytosis, predominantly neutrophilic leukocytosis.

Table I depicts the sites of infection in study participants. Majority (90%) of the cases did not have any prior history (in last 30 days) of hospitalization or treatment in a healthcare facility. Skin and soft tissue infections (SSTI) were the most common presentation (47%) followed by respiratory infections (37%). Abscess was the most common manifestation of SSTI found in 64% (*n*=30). Necrotizing fasciitis, the most severe form of SSTI was present in eight cases. Eight participants had complicated pneumonia. Septic arthritis (*n*=8) was more common than osteomyelitis (*n*=2).

Table II describes the antimicrobial susceptibility pattern of all isolates. Two-thirds of the isolates (*n*=62) were MRSA. All the isolates were uniformly susceptible to netilmicin, vancomycin and linezolid. There was no significant difference in the clinical data, anthropometry and biochemical parameters between MRSA and MSSA infected children.

The MIC values for vancomycin ranged from 0.25 µg/mL to 2 µg/mL. Two-third (65%) of the patients had high (>1 µg/mL) vancomycin MIC values. The median (IQR) of vancomycin MIC of MRSA was higher as compared to MSSA [1 (1,1.5) µg/mL vs 1 (0.44,1) µg/mL, *P*<0.001).

Table II Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolates (*N*=100)

Antibiotic	*MSSA(n=38)*	*MRSA (n=62)*
Cefoxitin,	38 (100)	0
Clindamycin[b]	33 (86.8)	36 (58.1)
Gentamicin[c]	37 (97.4)	34 (54.8)
Ampicillin[c]	30 (78.9)	1 (1.61)
Erythromycin[c]	19 (50)	3 (4.8)
Amikacin[b]	36 (94.7)	46 (74.2)
Netilmicin	38 (100)	62 (100)
Ciprofloxacin[a]	26 (68.4)	29 (46.8)
Cotrimoxazole	16 (42.1)	21 (33.9)
Vancomycin	38 (100)	62 (100)
Linezolid	38 (100)	62 (100)

Values in no. (%). MSSA-methicillin-sensitive S.aureus, MRSA-methicillin resistant S.aureus. [a]P<0.05; [b]P<0.01; [c]P<0.001.

A carrier state was found in 49 patients, out of which 32 were axillary carriers, 10 were nasal carriers and 7 were both nasal and axillary carriers, leading to 56 *S. aureus* isolates. Most (52, 93%) of these isolates were MRSA. In these 56 isolates, we compared their antibiotic susceptibility pattern to *S. aureus* isolated from site of infection in the same child. For 38 isolates, their methicillin (cefoxitin) susceptibility/resistance status was same as that of *S. aureus* isolated from site of infection. For 20 isolates, their antibiotic susceptibility pattern (for all antibiotics) was similar to the *S. aureus* isolated from infection site.

Ninety-six participants were discharged from hospital, three died and one left against medical advice. The mean (SD) duration of stay was 14.1 (5.5) days. The cause of death in three patients was sepsis, severe pneumonia and necrotizing fasciitis.

Poor recovery was present in 65 patients. On univariate analysis, severe stunting (HAZ score <-3 SD) (OR 4.54, 95%CI 1.4,14.4) and high vancomycin MIC (OR 7.0, 95%CI

Table III Factors Associated with Poor Recovery in *S. aureus* Infection (*N*=100)

Factor	*OR (95%CI)*	*aOR (95%CI)*
Age <6 mo	0.60 (0.25, 1.45)	0.39 (0.12,1.24)
Male gender	0.90 0.39, 2.05)	-
Presenting complaints		
Fever	0.57 (0.17, 1.92)	-
Chills	2.47 (0.89, 6.85)	2.38 (0.68,8.19)
Cough	0.92 (0.38,2.18)	
History		
Trauma	0.30 (0.10, 0.92)	0.39 (0.90,1.75)
Prior hospitalization	1.29 (0.31, 5.32)	-
Prior antibiotics	1.50 (0.49, 4.62)	-
Presence of abscess	0.57 (0.24,1.31)	0.65 (0.21,1.98)
BMI <-2SD	1.98 (0.85,4.60)	1.59 (0.54,4.69)
HFA *z*-score <-2SD	1.97 (0.85,4.58)	1.70 (0.61,4.73)
Hemoglobin< 10 g/dL	1.60 (0.62,4.15)	-
TLC > 15×10^9	1.46 (0.63,3.35)	-
Vancomycin MIC >1 µ/mL	7.07 (2.24,22.30)	5.34(1.56,18.5)
MRSA	1.37 (0.59,3.17)	-
Focus of infection		
Skin and soft tissue	0.66 (0.29,1.52)	-
Bone and joint	0.72 (0.21,2.48)	-
Respiratory	1.45 (0.61,3.47)	-
CNS	4.77 (0.57,39.82)	3.43 (0.33,34.4)
Sepsis without focus	1.41 (0.41,4.87)	-
Carrier state	0.51 (0.22,1.16)	0.47 (0.17,1.28)

BMI: body mass index, MRSA: methicillin resistant staphylococcal aureus, MIC: minimum inhibitory concentration, CNS: central nervous system, TLC – Total leukocyte count, HFA – Height for age.

2.2,22.3) were associated with higher odds of poor recovery, whereas history of trauma preceding the illness was associated with lesser odds of poor recovery (OR=0.3, 95%CI 0.1,0.9) (**Table III**). On logistic regression analysis, only high vancomycin MIC was a significant risk factor for poor recovery [aOR (95%CI) 5.3 (1.6,18.5); *P*=0.008].

DISCUSSION

In this study enrolling 100 children with culture positive staphylococcal infections, skin, soft tissue and respiratory tract were the most common sites of infection. MRSA was isolated in 62% isolates; 63% of MRSA were MDR *S. aureus*. Moreover, two-thirds (65%) of all *S. aureus* isolates had high MIC (>1 µg/mL) for vancomycin. Carrier state was present in almost half of the cases. High MIC for vancomycin was associated with poor recovery.

The global SENTRY surveillance program [8] conducted in 45 countries from North America, Latin America, Europe and Asia-Pacific region collected nosocomial and CA-*S. aureus* isolates from 1997-2016 and revealed that MRSA proportion of *S.aureus* had peaked till 2008, and declined since 2009. This reduction was consistent with several other regional and national surveillance programs during 2000-2010 [9]. This could be attributed to prioritizing MRSA infection prevention programs. However, in LMICs, the prevalence of MRSA seems to be increasing. In India, prevalence of MRSA was earlier reported to be 29-46% in hospital settings [10]. In the year 2013, Eshwara, et al. [7] documented MRSA in 54% of *S. aureus* bacteremia in children and adults, hospitalized in a tertiary care hospital in India. There are other recent reports of high prevalence of MRSA from developing countries [1,11]. Some of the possible reasons for high prevalence of MRSA in LMIC are irrational antibiotic prescription, socio-demographic factors like crowding and poverty resulting in circulation of resistant bacteriological strains in the community [12].

High MIC for vancomycin, a phenomenon referred to as 'MIC creep,' was first described in adults by Goldmann, et al. [13]; leading to concerns if vancomycin would be appropriate to treat invasive staphylococcal infection in presence of high vancomycin resistance and its association with poor outcome [14]. An earlier study in adults from North India documented that 50% of isolates from blood, skin and soft tissue infections had vanco-mycin MIC ≥1.5 µg/mL [15]. It has been seen that MIC creep phenomena is influenced by the type of *S. aureus* strain, type of patient population and storage of isolates [16].

We documented that high vancomycin MIC was significantly associated with poor recovery. Earlier systematic reviews [3,17] have also concluded that high MIC is associated with increased mortality and treatment failure. However, Pradhan, et al. [18], in a meta-analysis including 2955 patients from 13 reports, concluded that vancomycin MIC may not be the sole indicator of vanco-mycin treatment failure in MRSA and methodological differences, heterogenous population and differences in methods for estimation of drug susceptibility could be the other reasons.

Similar to this study, a high (42-82%) carrier state has also been reported in adults and children from other countries [19]. This may be of concern as colonized *S. aureus*, may act as reservoirs for future clinical infection and subsequent spread to the community. Lauderdale, et al. [20] observed that only nasal culture is not a sensitive marker of MRSA colonization, and if only nasal cultures are used, MRSA colonized patients are underestimated. Sending routine nasal and axillary swabs and eliminating carrier states may act as a step toward preventing community spread of MRSA.

The limitations of our study were the absence of long-term follow up to determine any recurrence of infections, lack of molecular characterization including *PVL* gene, and lack of epidemiological typing of the isolates. Another limitation in our study was that vancomycin MIC were determined by E-test which may provide 1-2 log dilutions higher MIC values than the reference broth microdilution test (BMD), which is the gold standard. However, E strip test is widely used as it is easy to perform and less cumbersome as compared to BMD. Also, E strip test when used with ATCC25923 *S. aureus* standard susceptible strain, as done in present study, maybe better correlated than BMD [4]. We, also, did not compare proportionate carriage of *S. aureus* in healthy and diseased population as we did not enrol any disease-free controls. The main strength of our study was availability of clinical data of patients till their discharge, which helped us to analyze the significance of antimicrobial susceptibility in a better way. We used E strip to detect vancomycin MIC, which is a more reliable method to determine the susceptibility of the organism to the glycopeptides.

We conclude that MRSA is the predominant staphylococcal strain in children hospitalized due to staphylococcal infections in our setting, and majority of them are multidrug resistant. Vancomycin may be used as a first line empiric antibiotic for serious staphylococcal infections and in those with documented MRSA. Vancomycin susceptibility testing can help in monitoring treatment especially in children with prolonged hospital stay or poor recovery. A higher proportion of MRSA, MDR and high vancomycin MIC in our group of patients with *S. aureus* infection is a serious concern with further risk of spread of resistant *S. aureus* infection in the community.

WHAT THIS STUDY ADDS?

- Methicillin-resistant *Staphylococcus aureus (*MRSA) was the predominant strain in children hospitalized due to staphylococcal infection acquired in the community settings.
- High MIC to vancomycin was associated with a poor outcome among *S. aureus* infected children.

Ethics clearance: Institutional Ethics Committee of Human Research, UCMS; No. IEC-HR/2017/32/99, dated Oct 17, 2017.
Contributors: KK: Conducted the study, data collection, literature review and drafted the manuscript; SK: data analysis, data interpretation, literature review, drafted and revising the manuscript. NPS: study design, provided laboratory support, supervised the study, provided critical inputs; PD: study design, supervised the study, literature review, provided critical inputs; PG: study design, supervised the study, literature review, provided critical inputs; DS: conceptualized the study, study design, literature review, data analysis, data interpretation, provided critical inputs. All authors approved the final manuscript.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Mahapatra MK, Mukherjee D, Poddar S, et al. Antibiotic sensitivity and clinico-epidemiological profile of staphylococcal infections. Indian Pediatr. 2016;53:923-4.
2. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) Group. Methicillin-resistant *Staphylococcus aureus* (MRSA) in India: prevalence and susceptibility pattern. Indian J Med Res. 2013;137:363-9.
3. Van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: a systematic review and meta-analysis. Clin Infect Dis. 2012;54:755-71.
4. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. CLSI document M100-S26. Clinical and Laboratory Standards Institute. 27th edition, 2017.
5. National Centre for Disease Control. National Treatment Guidelines for Antimicrobial use in Infectious Diseases. Version 1.0 (2016). Accessed on August 29, 2020. Available from: *http://pbhealth.gov.in/AMR_guideline 7001495889.pdfl*
6. Nebraska Medicine. Staphylococcus aureus Bloodstream Infection Treatment Guideline. Accessed August 29, 2020. Available from: *https://www.nebraskamed.com/sites/default/files/documents/for-providers/asp/staph-aureus-treatment.pdf.*
7. Eshwara VK, Munim F, Tellapragada C, et al. *Staphylococcus aureus* bacteremia in an Indian tertiary care hospital: Observational study on clinical epidemiology, resistance characteristics, and carriage of the Panton-Valentine leukocidin gene. Int J Infect Dis. 2013;17:e1051-5.
8. Daniel DJ, Pfaller MA, Shortridge D, et al. Trends in antimicrobial susceptibilities among *Staphylococcus aureus* from the SENTRY antimicrobial surveillance program. Open Forum Infect Dis. 2019;6: S47-53.
9. Johnson AP, Davies J, Guy R, et al. Mandatory surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia in England: The first 10 years. J Antimicrob Chemother. 2012; 67:802-9.
10. Patel AK, Patel KK, Patel KR, et al. Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. J Assoc Physicians India. 2010; 58:37-40.
11. Dharmapalan D, Shet A, Yewale V, et al. High reported rates of antimicrobial resistance in Indian neonatal and pediatric blood stream infections. J Pediatric Infect Dis Soc. 2017; 6: e62-8.
12. Andreatos N, Shehadeh F, Pliakos EE, et al. The impact of antibiotic prescription rates on the incidence of MRSA bloodstream infections: A county-level, US-wide analysis. Int J Antimicrob Agents. 2018;52:195-200.
13. Goldman JL, Harrison CJ, Myers AL, et al. No Evidence of vancomycin minimum inhibitory concentration creep or heteroresistance identified in pediatric *Staphylococcus aureus* Blood Isolates. Pediatr Infect Dis J. 2014;33:216-8.
14. Moellering RC Jr, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. J Clin Microbiol. 2004;42:2398-402.
15. Mewara A, Gautam V, Kaur H, et al. In vitro evaluation of antibiotics for methicillin resistant *Staphylococcus aureus* from North India. Indian J Med Res. 2014;139:319-22.
16. Sharma R, Hammerschlag MR. Treatment of methicillin-resistant staphylococcus aureus (MRSA) infections in children: A Reappraisal of vancomycin. Curr Infect Dis Rep. 2019;21:37.
17. Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical Staphylococcus aureus isolates ('the MIC Creep'): Implications for therapy. F1000 Med Rep. 2012; 4:4.
18. Kale-Pradhan PB, Mariani NP, Wilhelm SM, et al. Meta-analysis: Vancomycin treatment failures for MRSA bacteremia based on MIC determined by E-test. J Pharm Technol. 2016; 32:65-70.
19. Saxena S, Singh K, Talwar V. Methicillin-resistant *Staphylococcus aureus* prevalence in community in the East Delhi area. JPN J Infect Dis. 2003;56:54-6.
20. Lauderdale, TL, Wang, JT, Lee, et al. Carriage rates of methicillin-resistant Staphylococcus aureus (MRSA) depend on anatomic location, the number of sites cultured, culture methods, and the distribution of clonotypes. Eur J Clin Mic Infec Dis. 2010;29:1553-59.