

1 Community-associated and health care-associated methicillin-
2 resistant *Staphylococcus aureus* in children: a microbiological
3 and epidemiological study
4
5
6

7 **Abstract**

8 Aims: Novel methicillin-resistant *Staphylococcus aureus* have been causing infections in the
9 community and are now invading hospitals. In this study we aimed to determine, using
10 epidemiological and microbiological parameters, the characteristics of circulating *S. aureus*
11 clinical isolates.

12 Methods: From July 2009 to April 2012, *S. aureus* isolates from children hospitalized in Santa
13 Casa de São Paulo, a tertiary care-center in São Paulo, Brazil, were included. All isolates grew
14 in cultures from sterile sites and we included only one isolate per patient.

15 Results: Fifty-five isolates were included during the study period, 47 from blood, six from
16 abscesses, one from pleural fluid and one from spinal fluid. Among these isolates, 34 were
17 methicillin susceptible *S. aureus* (MSSA) and 21 were methicillin-resistant *S. aureus* (MRSA).
18 Eleven patients were excluded (5 MSSA and 6 MRSA) because clinical charts were not available
19 for review, reducing the total to 29 MSSA and 15 MRSA isolates. After searching for risk factor
20 for healthcare-associated infections, 11 of the 15 MRSA isolates were epidemiologically
21 considered health care-associated MRSA (HCA-MRSA) and 4 community-associated MRSA (CA-
22 MRSA). Using the microbiological classification (multiresistance), five were considered as HCA-
23 MRSA and 10 were CA-MRSA. Interestingly, of the 11 isolates considered as epidemiological
24 HCA-MRSA (presence of any risk factor), six had a microbiological profile (non-multiresistant)
25 consistent with CA-MRSA circulating clones.

26 Conclusion: Our results clearly show that the boundaries between CA-MRSA and HCA- are
27 increasingly difficult to determine.

28
29 **Keywords**

30 *Staphylococcus aureus*; MRSA; antimicrobial resistance; oxacillin
31
32

33 Introduction

34 *Staphylococcus aureus* commonly colonizes humans in different anatomical sites. Traditionally
35 it is more frequently found in the nose. However, recent evidence suggests that oropharynx
36 could be an even more important site of colonization [1]. Some specific populations, as cystic
37 fibrosis patients, are more commonly colonized than the general population [2]. Depending on
38 host and pathogen factors, *S. aureus* may cause infection. Infectious syndromes include, but
39 are not limited to respiratory, skin and soft tissue, osteoarticular and bloodstream infections.
40 *S. aureus* is an important etiologic agent of both community- and healthcare-associated
41 infections [1-3].

42 Resistance to antimicrobial agents has complicated the treatment of *S. aureus* infections. After
43 penicillin resistance, methicillin resistance disseminated worldwide, first in the hospitals and
44 eventually in the community [3-6]. These new community-associated methicillin resistant *S.*
45 *aureus* (CA-MRSA) clones, described originally in the 1990s, have some differences when
46 compared to the traditional health care-associated (HCA-MRSA) clones. CA-MRSA are usually
47 resistant to less antimicrobial agents, usually only to beta-lactams and macrolides (and
48 increasingly to clindamycin) [3-5]. These are the phenotypical implications of the presence of
49 different Staphylococcal cassette chromosome *mec* (SCC*mec*) types. SCC*mec* is a mobile
50 genetic element that includes *mecA* gene, responsible for methicillin resistance in *S. aureus*.
51 CA-MRSA most commonly carry SCC*mec* types IV or V [4-6]. Some of these clones also have a
52 specific virulence profile, frequently producing Panton-Valentine leukocidin and other typical
53 virulence factors⁴⁻⁷.

54 These new clones have also been reported causing nosocomial infections, substituting, at least
55 in part, traditional health-care associated MRSA clones. In Brazil, although CA-MRSA is still not
56 common, clones carrying SCC*mec* IV have been increasingly found in different hospitals [8-12].
57 Resistance to oxacillin is common in the bacterial isolates from hospitals, varying from 30-60%
58 [9-12]. In the present study, we aimed to characterize the *S. aureus* clinical isolates regarding
59 their epidemiological (presence or absence of risk factors for health-care associated infections)
60 and microbiological (antimicrobial multiresistance or non-multiresistance as a surrogate for
61 SCC*mec* type) profiles.

62

63 Methods

64 From July 2009 to April 2012, *S. aureus* isolates from children hospitalized in Santa Casa de São
65 Paulo, a tertiary care-center in São Paulo, Brazil, were included. All isolates grew in cultures
66 from previously sterile sites and we included only one isolate per patient. Bacterial
67 identification was conducted using traditional biochemical methods (Gram stain, catalase,
68 coagulase and DNase) and antimicrobial susceptibility testing was performed according to
69 current Clinical and Laboratory Standards Institute criteria [13].

70 Methicillin-resistant isolates resistant to four or more different classes of antibiotics were
71 considered multiresistant (HCA-MRSA by microbiological classification), whereas isolates

72 resistant to three or less classes were considered non-multiresistant (CA-MRSA by
73 microbiological classification) [4-6].

74 Clinical charts were reviewed and searched for the presence of any risk factors for health-care
75 associated infections, including hospitalization within the previous year, chronic disease that
76 implicates in frequent contact (≥ 6 /year) with hospital environment, or current hospitalization
77 for at least 48 hours. Isolates were considered as HCA-MRSA (epidemiologically) if any of these
78 risk factors were present, and were considered as CA-MRSA (epidemiologically) if they were all
79 absent [4-6, 9-12]. The study was approved by the institutional ethics committee.

80

81 **Results and Discussion**

82 Fifty-five isolates were included during the study period, 47 from blood, six from abscesses,
83 one from pleural fluid and one from spinal fluid. Regarding oxacillin resistance, 34 were MSSA
84 and 21 were MRSA. Eleven patients were excluded (5 MSSA and 6 MRSA) because clinical
85 charts were not available for review, reducing the total to 29 MSSA and 15 MRSA isolates.

86 After searching for risk factor for healthcare-associated infections, 11 of the 15 MRSA isolates
87 were epidemiologically considered HCA-MRSA and 4 CA-MRSA. Using the microbiological
88 classification (multiresistance), five were considered HCA-MRSA and 10 were considered CA-
89 MRSA. Interestingly, of the 11 isolates considered epidemiological HCA-MRSA (presence of one
90 or more a risk factors), six had a microbiological profile (non-multiresistant) consistent with
91 CA-MRSA circulating clones.

92 Although these novel *SCCmec* type IV MRSA clones were first reported in the community,
93 there is increasing evidence that they are replacing traditional hospital circulating clones [8-
94 10]. In Brazil, the Brazilian clone (*SCCmec* type III) have been disseminated nationally in
95 hospitals, but recent evidence shows that different clones carrying a type IV cassette are
96 disseminating as a cause of healthcare-associated infections [11,12].

97 Although the number of isolates included in our study is low, and we have not performed
98 molecular typing, phenotypic susceptibility testing served as a surrogate for the *SCCmec* type,
99 since we have previously and consistently reported the strong correlation between these
100 parameters [11,12]. In addition, the results in the present study clearly show that the
101 boundaries between CA-MRSA and HCA- MRSA are increasingly difficult to determine.
102 Nosocomial isolates cause infection in patients without risk factors and community isolates
103 invade the hospital causing health-care associated infections. In these challenging
104 epidemiological and microbiological situation, knowledge of local and global circulating clones
105 is paramount to ensure the appropriate preventive and therapeutic approaches.

106

107 **References**

1. Bádue Pereira MF, Mimica MJ, Carvalho RLB, Scheffer DK, Berezin EN. High rate of *Staphylococcus aureus* oropharyngeal colonization in children. J Infect. 2012; 64:338-340.
2. Mimica MJ, Carvalho RL, Berezin EN, Damaceno N, Caiaffa-Filho HH. Comparison of five methods for oxacillin susceptibility testing of *Staphylococcus aureus* isolates from cystic fibrosis patients. Rev Inst Med Trop Sao Paulo. 2012; 54:305-306.
3. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A. 2002;99:7687-92.
4. Weber JT. Community-associated methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 2005;41:S269-72.
5. Hultén KG, Kaplan SL, Gonzalez BE, Hammerman WA, Lamberth LB, Versalovic J, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. Pediatr Infect Dis J. 2006 Apr;25(4):349-53.
6. Qiao Y, Dong F, Song W, Wang L, Yang Y, Shen X. Hospital- and community-associated methicillin-resistant *Staphylococcus aureus*: a 6-year surveillance study of invasive infections in Chinese children. Acta Paediatr. 2013;102:1081-6.
7. Mimica MJ. Pantón-Valentine leukocidin-positive *Staphylococcus aureus* skin infections. Clin Infect Dis. 2012;54:1517-8
8. Gonzalez BE, Rueda AM, Shelburne SA 3rd, Musher DM, Hamill RJ, Hultén KG. Community-associated strains of methicillin-resistant *Staphylococcus aureus* as the cause of healthcare-associated infection. Infect Control Hosp Epidemiol. 2006; 27:1051-6.
9. Trindade PA, Pacheco RL, Costa SF, Rossi F, Barone AA, Mamizuka EM, et al. Prevalence of SCCmec type IV in nosocomial bloodstream isolates of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol. 2005; 43:3435-7.
10. Vidal PM, Trindade PA, Garcia TO, Pacheco RL, Costa SF, Reinert C, et al. Differences between "classical" risk factors for infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and risk factors for nosocomial bloodstream infections caused by multiple clones of the staphylococcal cassette chromosome mec type IV MRSA strain. Infect Control Hosp Epidemiol. 2009;30:139-45.
11. Mimica, M.J.; Berezin, E.N.; Carvalho, R.B. Healthcare associated PVL-negative methicillin-resistant *Staphylococcus aureus* with SCCmec type IV. Pediatr Infect Dis J. 2009;28:934.
12. Mimica MJ, Berezin EN, Damaceno N, Carvalho RB. SCCmec Type IV, PVL-Negative, Methicillin-Resistant *Staphylococcus aureus* in Cystic Fibrosis Patients from Brazil. Curr Microbiol. 2011;62:388-90.
13. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement, M100-S23. Wayne, PA, USA: Clinical and Laboratory Standards Institute. 2013.

151

152

153

154