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**Epidemiology of borderline oxacillin-resistant
Staphylococcus aureus in pediatric cystic fibrosis.**

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4 1 **Epidemiology of borderline oxacillin-resistant *Staphylococcus***
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7 2 ***aureus* in pediatric cystic fibrosis.**
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10 3

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27 20 **Keywords:** methicillin resistant *Staphylococcus aureus* (MRSA), *mecA*, pulsed-field gel

28 21 electrophoresis.

29 22 **Abbreviated title.** BORSA in pediatric cystic fibrosis

30 23

24 Summary

25 A single-centre retrospective study was undertaken in children with cystic fibrosis (CF)
26 to evaluate 1) risk factors for acquisition; 2) molecular epidemiology; and 3) impact on
27 disease progression of borderline oxacillin-resistant *Staphylococcus aureus* (BORSA)
28 versus *mecA*-positive methicillin-resistant *Staphylococcus aureus* (MRSA). The study
29 comprised of 1) identification of all children with at least one respiratory specimen
30 positive for either BORSA or MRSA during the study period; 2) compilation of relevant
31 clinical and epidemiological data from 12-month period leading up to first positive
32 (index) culture; 3) microbiological and molecular characterisation of index isolates and 4)
33 measurement of subsequent clinical outcome. Thirty-eight children were identified with
34 at least one positive isolate; 4 were excluded due to insufficient clinical or laboratory
35 data. Eighteen children (53%) grew BORSA in their index culture. Children who
36 acquired BORSA only (n=16) were more likely to have had prior MSSA colonization
37 (p<0.0001). Usage of oral cephalexin (p<0.01) and inhaled tobramycin (p<0.03) prior to
38 index culture was significantly and independently associated with acquisition of BORSA.
39 The majority of BORSA isolates were hyper β -lactamase producers and susceptible to a
40 greater range of antibiotics. Strain relatedness was not evident within the BORSA group.
41 There was no difference in disease progression between the two groups. This is the first
42 study to demonstrate that a significant proportion of *S. aureus* isolates with methicillin
43 resistance in the CF population are BORSAs that lack *mecA*. Antibiotic pressure may
44 lead to the development of BORSA in CF patients. Prospective studies are needed to
45 assess its clinical impact.

46 Introduction

47
48 Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as an important
49 respiratory pathogen in the cystic fibrosis (CF) population. The US CF registry reported a
50 significant rise in MRSA infection among CF patients, from 2.1% in 1996 to 21.2% in
51 2007¹. MRSA infection has been associated with accelerated deterioration in pulmonary
52 function², increased hospitalization³, impaired growth, increased antibiotic usage⁴ and
53 increased mortality in CF patients⁵.

54 According to the Clinical Laboratory Standards Institute (CLSI), methicillin
55 resistance in *S. aureus* is defined as having an oxacillin minimum inhibitory
56 concentration (MIC) > 4 µg/ml⁶. The primary mechanism of resistance for MRSA is the
57 production of an altered penicillin binding protein (PBP2a) with reduced affinity for
58 methicillin that is encoded by the *mecA* gene⁷. We have identified a group of CF patients
59 infected with borderline resistant *S. aureus* (BORSA) isolates that are characterized by
60 oxacillin MICs at or just above the susceptibility breakpoint of 4 µg/ml and lack the
61 *mecA* gene⁷. The implications of isolating BORSA in a respiratory culture from CF
62 patients have not previously been explored. The aim of this study was therefore to
63 compare the risk factors for acquisition, the molecular epidemiology and subsequent
64 clinical impact on disease progression of BORSA infection in comparison with *mecA*-
65 positive MRSA infection in pediatric CF patients.

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67

68 **Materials and Methods**

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70 Study design and patient population

71 This was a single-centre retrospective comparative study of pediatric CF patients
72 whose care was primarily based at the CF clinic at the Hospital for Sick Children,
73 Toronto between 1992 and 2007. The clinic currently cares for approximately 280
74 children up to the age of eighteen. Respiratory samples (expectorated sputum or
75 oropharyngeal swabs) were sent from all children attending the clinic to the microbiology
76 laboratory for culture at every 3-month visit and during acute pulmonary exacerbations
77 (APEs). Patients with one or more respiratory specimens positive for *S. aureus* with
78 methicillin resistance (either BORSA or MRSA) were included in the analysis. Cases
79 with three or more specimens positive for BORSA or MRSA during the study period
80 were considered persistently colonized². The study was approved by the Hospital for
81 Sick Children's Research Ethics Board.

82

83 Data collection

84 Patients with BORSA or MRSA isolated from their respiratory specimens were
85 identified using the microbiology database, and cross-referenced with the CF clinical
86 database. Date of the first BORSA or MRSA-positive respiratory culture (index culture)
87 for each patient was identified. The medical and laboratory records for each patient were
88 examined using a standardized data collection form. Baseline patient characteristics were
89 assessed at the date of index culture, and risk factors for acquisition of BORSA or MRSA
90 infection were assessed over the twelve-month period prior to the index culture. Impact

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3 91 on clinical progression was assessed by examination of the time period from index
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5 92 culture until discharge from the clinic or until December 31st 2008, whichever date was
6
7
8 93 later. Data collected included information on patients' demographics, pulmonary function
9
10 94 tests (PFTs), respiratory cultures, hospital admissions, acute pulmonary exacerbations
11
12 95 and antibiotic use. An **acute pulmonary exacerbation** was defined as a change in
13
14 96 pulmonary status considered by the attending physician to require antibiotic treatment,
15
16 97 either oral or intravenous. An "antibiotic day" was defined as one day for each antibiotic
17
18 98 used, for example a 14-day course of two antibiotics was defined as 28 "antibiotic days".
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20 99 The purpose of this distinction was to capture more complex antibiotic regimens that
21
22 100 might be employed to treat MRSA infected patients. Patients were classified as being on
23
24 101 oral cephalexin if they were prescribed cephalexin at the time of index culture of at least
25
26 102 one month's duration (in all instances, this occurred in the absence of signs or symptoms
27
28 103 of a pulmonary exacerbation). Patients were classified as being on inhaled tobramycin if
29
30 104 they were prescribed inhaled tobramycin at the time of index culture of at least one
31
32 105 month's duration (**for previous *P. aeruginosa* infection**). Patients were classified as being
33
34 106 on oral ciprofloxacin if they were prescribed at least one course of ciprofloxacin (in all
35
36 107 instances, this occurred in the presence of signs or symptoms of a pulmonary
37
38 108 exacerbation). Patients were categorized as co-colonized with other CF pathogens using
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40 109 previously defined criteria ⁸. Only patients with at least 6 months of follow-up after the
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42 110 index culture were included in the analysis of outcome measures.
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3 114 Microbiology
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5 115 **Specimen Processing**
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8 116 Respiratory specimens from CF patients were screened for MRSA using media
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10 117 containing 2 µg/ml of oxacillin: an in-house mannitol salt agar (MRSA agar base,
11
12 118 Acumedia, Lansing, MI, and 1% D-mannitol, Difco, Detroit, MI) supplemented with 1%
13
14 119 thymidine and 2 µg/ml of oxacillin (both from Sigma, St. Louis, MO) was used from
15
16 120 1992 to 2001, and Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid, Nepean,
17
18 121 Ontario, Canada) was used from 2002 onward. Plates were incubated at 35°C aerobically
19
20 122 and observed for the presence of growth at 24 hours and at 48 hours. Suspicious colonies
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22 123 were identified as *S. aureus* by Gram stain, catalase, slide coagulase, and tube coagulase
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24 124 tests.
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31 125
32 126 **Antimicrobial Susceptibility**
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34 127 Antimicrobial susceptibility of *S. aureus* isolates was tested by a combination of
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36 128 oxacillin 6µg/ml screen plate, disk diffusion^{6,9} and an automated system (Vitek, bio-
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38 129 Merieux, St. Laurent, Quebec, from 1992 to Nov 2002, and the BD Automated Phoenix
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41 130 System, BD Diagnostic Systems, Sparks, MD from Nov 2002 onward). Inducible
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43 131 resistance to clindamycin was detected by disk diffusion using the D-zone test^{6,9}. In
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45 132 addition, each strain was examined for the presence of PBP2a by latex agglutination
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47 133 (PBP2' test kit, Oxoid, Hants, UK). Organisms that were negative for PBP2a by latex
48
49 134 agglutination but grew on the oxacillin screen plate were sent to the reference laboratory
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51 135 (Central Provincial Health Laboratory, Ontario) for minimum inhibitory concentration
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53 136 (MIC) by agar dilution and polymerase chain reaction (PCR) for the detection of the
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3 137 *mecA* gene. An isolate was deemed as BORSA if the oxacillin MIC was $\geq 4 \mu\text{g/ml}$,
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5 138 PBP2a was absent and the *mecA* PCR was negative⁷. An isolate was deemed as MRSA if
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8 139 the oxacillin MIC was $\geq 4\mu\text{g/ml}$, the latex agglutination for PBP2a was positive and/or the
9
10 140 *mecA* PCR was positive⁶.
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142 **β -Lactamase Production**

143 All BORSAs were tested for the production of β -lactamase as a potential
144 mechanism of oxacillin resistance by disk diffusion with ampicillin (10 μg) and
145 amoxicillin-clavulanic (20 μg / 10 μg) disks as previously described (13). An isolate was
146 deemed as hyper β -lactamase producing if the ampicillin zone size was resistant and the
147 amoxicillin-clavulanic zone size was susceptible, suggesting borderline resistance was
148 secondary to large amounts of β -lactamase⁹(13).
149

150 **Molecular Characterization**

151 The *mecA* and *nuc* gene were co-amplified with a multiplex real time PCR as
152 described previously¹⁰. The presence of Panton-Valentine leukocidin was examined by
153 amplification of the *lukF-PV* and *lukS-PV* genes using primer and protocols previously
154 described¹¹. Pulsed-field gel electrophoresis (PFGE) was conducted using *SmaI* as per
155 manufacturer protocol (Bio-rad Laboratories, Hercules, CA) on all newly identified
156 MRSA strains. Electrophoretically-generated DNA profiles were analyzed using the
157 criteria proposed by Tenover et al to determine strain relatedness¹².
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3 160 Statistical analysis
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5 161 Descriptive statistics, frequency distributions and percentages were calculated for
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8 162 the outcome variables and other covariates of interest. Normality assumption test was
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10 163 carried out for the continuous variables using the Kolmogorov-Smirnov test. Student's t-
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12 164 test or Kruskal-Wallis test (if normality assumption failed) was conducted for continuous
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14 165 variables within the two groups. The Chi-square or Fisher's exact test, as appropriate,
15
16 166 was carried out for the dichotomous variables to assess the association with the two
17
18 167 groups. To analyze independent risk factors, a multivariate analysis using a logistic
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20 168 regression model was applied. A p-value of <0.05 was considered statistically significant.
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22 169 The statistical programme SAS (version 9.1; SAS Institute Inc., Cary, NC) was used.
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3 171 **Results**

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8 173 Patient population

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11 Between 1992 and 2007, 38 patients had at least one respiratory specimen
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13 positive for either BORSA or MRSA. Of these, three were excluded from our analysis
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15 because their care was not primarily based at our hospital, thus we had no clinical data
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17 for these patients. In addition, *mecA* PCR could not be performed on one isolate
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19 retrospectively therefore 34 patients were included in the study. Of these 34 patients, two
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21 patients were initially colonized with *mecA*- negative and subsequently *mecA*-positive
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23 strains and were excluded from the final statistical analysis as it was unclear how to
24
25 categorize them in terms of patient characteristics and outcomes. Ultimately 16 patients
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27 with *mecA* positive MRSA and 16 with *mecA* negative BORSA on the index culture were
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29 compared for baseline characteristics. For the longitudinal assessment, 15 patients in each
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31 group were compared since one patient in each group did not have follow-up data for at
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33 least 6 months.
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42 187 Patient characteristics and risk factors for BORSA and MRSA

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44 The two groups were similar in terms of baseline characteristics (Table 1). There
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46 was no difference in pulmonary function or rate of decline of pulmonary function in the
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48 year prior to the index culture. In addition, there was no difference in the number of acute
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50 **pulmonary exacerbations**, hospital admissions or overall antibiotic use for the treatment
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52 of acute **pulmonary exacerbations**. A significantly greater proportion of BORSA infected
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54 patients were colonized with MSSA in the year prior (94% vs 25%, $p < 0.0001$). Likewise,
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3 194 a higher proportion of BORSA infected patients received oral antibiotics (cephalexin)
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5 195 (75% vs 25%, p=0.005).
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8 196 Mean duration of therapy among those patients on cephalexin was 337 days (range 30-
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10 197 365 days) in the BORSA group and 311 days (range 150-365 days) in the MRSA group;
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12 198 cephalexin treatment was instituted not for pulmonary exacerbation but as treatment for
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15 199 MSSA identified on routine sputum cultures. Furthermore, a significantly greater
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17 200 number of BORSA infected patients had received a course of oral ciprofloxacin (31% vs
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19 201 0%, p=0.043) or were on inhaled antibiotics (tobramycin) (88% vs 44%, p=0.009) in the
20
21 202 year prior to the index culture than patients with MRSA. No inter-group difference was
22
23 203 detected in any of the other co-colonizing respiratory pathogens. Patients with BORSA
24
25 204 strains had significantly more clinic visits in the year prior to first isolate (n=7.94) than
26
27 205 patients with *mecA*-positive strains (n=4.69, p=0.049) although this was not a significant
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29 206 risk factor in the logistic regression model
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34 207 A logistic regression model was applied to determine the independence of
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36 208 epidemiological and clinical variables that were correlated with BORSA infection.
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38 209 Colonization with MSSA and use of oral cephalexin were significantly associated as 68%
39
40 210 of patients with MSSA were treated with cephalexin and 81% of patients treated with
41
42 211 cephalexin were colonized with MSSA (p<0.05). The use of oral cephalexin (regression
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44 212 coefficient (β)=1.15, SD=0.47, p value=0.015) and inhaled tobramycin (regression
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46 213 coefficient (β)=1.16, SD=0.53, p value=0.028) in the year prior to index culture were
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48 214 significantly and independently associated with BORSA infection.
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55 216 Microbiological characteristics of BORSA and MRSA isolates
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3 217 The microbiological characteristics of the BORSA isolates are illustrated in Table
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5 218 2. Fourteen of the 16 isolates had oxacillin MICs at or just above the breakpoint (4 to 8
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8 219 $\mu\text{g/ml}$) but 2 isolates had MICs of 16 $\mu\text{g/ml}$. All of the BORSA isolates were negative for
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10 220 PBP2a by latex agglutination and were *mecA* negative by PCR. Twelve of the 16 BORSA
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12 221 isolates were hyper β -lactamase producers. This was shown by resistance to ampicillin by
13
14 222 disk diffusion that was reverted to susceptible with the addition of clavulanic acid, a β -
15
16 223 lactamase inhibitor. The remaining 4 isolates had ampicillin zone size that were
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18 224 unchanged by addition of clavulin by disk diffusion.
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22 225 In comparison to the MRSA isolates, a significantly greater proportion of the
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24 226 BORSA isolates were susceptible to both erythromycin (81% vs 13%, $p < 0.0001$) and
25
26 227 clindamycin (94% vs 31%, $p < 0.001$). None of the BORSA isolates were D-test positive
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28 228 but 5 (45%) of the MRSA isolates were D-test positive ($p = 0.043$). All of the BORSA
29
30 229 and MRSA isolates were susceptible to trimethoprim-sulphamethoxazole. None of the
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32 230 BORSA isolates tested produced PVL. Three of the 16 MRSA isolates were identified as
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34 231 USA300 epitypes, and produced PVL.
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41 232 Relatedness of BORSA and MRSA isolates

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43 234 Pulsed field gel electrophoresis (PFGE) revealed that none of the BORSA strains
44
45 235 were related by the Tenover criteria¹³ (Figure 1). Within the MRSA group, we found
46
47 236 four separate instances of strain relatedness during the course of the study: a pair of
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49 237 siblings shared an indistinguishable strain, four patients shared possibly related strains,
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51 238 two patients shared a closely related strain with a third patient sharing a possibly related
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3 239 strain and, finally, three patients (including a pair of siblings) shared an indistinguishable
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5 240 strain.
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10 242 Clinical outcomes of patients

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13 243 To compare the clinical impact of BORSA versus MRSA infection on disease
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15 244 progression, we assessed 99.7 patient-years of follow-up after the index culture with a
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17 245 comparable mean duration of follow up of 3.37 years in the MRSA group and 3.27 years
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20 246 in the BORSA group ($p=0.507$) (Table 3). Patients infected with MRSA were more
21
22 247 likely to have persistent infection at the end of follow up than patients with BORSA
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24 248 infection (80% vs 53%) but this was not statistically significant ($p=0.121$). The annual
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26 249 decline in percent predicted forced expiratory volume in 1 second (FEV_1), was greater
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28 250 among the MRSA infected patients (5.8%) than those infected with BORSA (2.8%)
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30 251 although this difference was not significant ($p = 0.77$). Neither was there a significant
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32 252 difference in the number of acute pulmonary exacerbations, hospital admissions, inpatient
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34 253 days or clinic visits. One patient in the MRSA group died, and another progressed to lung
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36 254 transplant. Three patients in the BORSA group had a lung transplant during the study
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3 257 **Discussion**
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8 259 This is the first study to show that a significant proportion (50%) of *S. aureus*
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10 260 isolates with methicillin resistance infecting CF patients are BORSA which lack the
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12 261 *mecA* gene, the main genetic determinant of methicillin resistance in *S. aureus*⁷. The
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14
15 262 present study demonstrates that antibiotic use in CF patients colonized with MSSA is a
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17 263 risk factor specifically for infection with BORSA. Previous studies have shown that
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19 264 ciprofloxacin and cephalosporin use is a risk factor for acquisition of MRSA in CF
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21 265 patients¹⁴ but, to our knowledge, no other study has examined the differential risk factors
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23 266 for BORSA compared to MRSA acquisition.
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27 267 Transmission of traditional MRSA between patients^{15,16}, within the community¹⁷
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29 268 and even within households¹⁸ has been well described in the CF population. Evidence of
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31 269 person to person transmission of *mecA*-positive MRSA was also present in this study
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33 270 with shared strains among CF patients and siblings. The mechanism of resistance is
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35 271 relevant to how CF patients acquire MRSA as the *mecA* gene is absent in its entirety from
36
37 272 MSSA^{19,7}. It is believed to have originated from another staphylococcus species, such as
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39 273 *Staphylococcus sciuri*, and been transposed into MSSA²⁰. Although horizontal transfer
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41 274 of *mecA* between a *S. epidermidis* and MSSA isolate within the same patient has been
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43 275 described²¹, transmission of *mecA*-positive MRSA isolates between individuals is likely
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45 276 more common as there are a well defined number of *mecA*-positive MRSA clones
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47 277 worldwide²².
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53 278 Our data would suggest that CF patients may acquire *mecA*-negative BORSA
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55 279 isolates in a different way. In contrast to MRSA isolates, BORSA isolates had unique
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3 280 PFGE patterns and appeared not to be shared between patients in our study. CF patients
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5 281 colonized with MSSA and exposed to oral cephalexin or inhaled tobramycin (which can
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7 282 act synergistically with β -lactam antibiotics against *S. aureus*) were at significantly
8
9 283 increased risk of acquiring BORSA infection. This suggests that BORSA isolates may
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11 284 arise from endogenous MSSA isolates that develop other mechanisms of resistance to
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13 285 methicillin in response to antibiotic pressure. Previous studies have shown that *S. aureus*
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15 286 strains with borderline susceptibility to oxacillin have a significant drop in the penicillin
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17 287 MICs to the susceptible range with the addition of a β -lactamase inhibitor²³. This
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19 288 predicts the response of oxacillin MICs to the addition of a β -lactamase inhibitor and
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21 289 suggests that a large amount of β -lactamase production is the mechanism of resistance to
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23 290 oxacillin in the majority of BORSA, as observed in 12 of 16 isolates in our study.
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25 291 However, in the 4 remaining BORSA strains where the ampicillin zone size did not
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27 292 change significantly with the addition of clavulanic acid, the mechanism of methicillin
28
29 293 resistance is unknown. In the aforementioned study, the authors described *S. aureus*
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31 294 strains with intermediate MICs to oxacillin that remained the same in the presence of
32
33 295 clavulanic acid. The authors suggested that oxacillin resistance was due to factors other
34
35 296 than β -lactamase inactivation²³. It is possible that it is due to a modification of “normal”
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37 297 penicillin-binding proteins (PBPs) such as PBP 1, PBP 2 and PBP 4 as was previously
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39 298 shown in clinical BORSA isolates by Tomasz et al²⁴. Exposure of susceptible
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41 299 staphylococci to increasing concentrations of β -lactam antibiotics in the laboratory is
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43 300 known to cause PBP alterations associated with increased MICs²⁵. In this study,
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45 301 persistent antibiotic pressure may have similarly modified PBPs in endogenous MSSA
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47 302 isolates colonizing CF patients and resulting in the development of *mecA*-negative, non-
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3 303 hyper β lactamase producing BORSA isolates. Thus, in the prevention of the emergence
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5 304 of BORSA in CF patients, other infection control measures, such as patient segregation,
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8 305 may not be as important as the judicious use of antibiotics.
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10 306 In our study, the majority of patients treated with oral cephalexin were colonized
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12 307 with MSSA. Although there were no signs and symptoms of a pulmonary exacerbation at
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14 308 the time, we are limited in a retrospective study to the information that is recorded in the
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16 309 medical charts. Their antibiotic treatment was thus likely driven by their MSSA positive
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18 310 respiratory cultures and/or more subtle symptoms. Primary prophylaxis for *S. aureus* (the
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20 311 treatment of all newly diagnosed CF patients with antistaphylococcal agents irrespective
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22 312 of respiratory culture results) is not routinely practiced in North America due to concerns
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24 313 of *P. aeruginosa* acquisition^{26,27}. A systematic review of prophylactic antibiotics in
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26 314 cystic fibrosis, however, suggests that *P. aeruginosa* acquisition in this context is
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28 315 associated less frequently with narrow spectrum anti-staphylococcal antibiotics such as
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30 316 flucloxacillin compared with broader spectrum anti-staphylococcal antibiotics such as
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32 317 cephalexin²⁸. In our study, although patients treated with oral cephalexin were not
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34 318 receiving prophylaxis against *S. aureus*, they were receiving prolonged courses of
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36 319 cephalexin for MSSA detected on routine culture of the respiratory tract. This prolonged
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38 320 cephalexin use was identified as an independent risk factor for the acquisition of BORSA
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40 321 isolates and occurred recently, primarily in the last 5 years of the study.
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48 322 The clinical significance of BORSA infection in CF patients is unknown. Our
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50 323 study lacked a sufficient sample size and is not powered to clearly delineate the clinical
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52 324 impact of BORSA infection. Previous studies have reported accelerated deterioration in
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54 325 pulmonary function², increased hospitalization³, impaired growth, increased antibiotic
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3 326 usage⁴ and increased mortality⁵ in CF patients infected with MRSA. However, these
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6 327 studies are limited by a lack of information on the laboratory methodology of identifying
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8 328 MRSA and thus it is not known to what degree these effects are attributable to BORSA or
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10 329 MRSA.

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13 330 Detection of BORSA isolates may also influence the choice of antibiotics in the
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15 331 treatment of these infections in the setting of a pulmonary exacerbation. In our study,
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17 332 BORSA isolates were more susceptible to erythromycin and clindamycin than the MRSA
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19 333 isolates. This finding is not surprising as the *mecA* gene is located on a mobile genetic
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21 334 element (the Staphylococcal Cassette Chromosome *mec*: SCC*mec*) which can contain
22
23 335 additional drug resistance genes²². Although earlier studies suggested that infections due
24
25 336 to *mecA* negative BORSA isolates could be treated with β -lactam antibiotics^{29,30}, more
26
27 337 recent cases report failure of cloxacillin and even vancomycin in the treatment of
28
29 338 endocarditis caused by BORSA, depending on the mechanism of methicillin resistance
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31 339^{31,32}. This has implications for how respiratory specimens from CF patients should be
32
33 340 screened for MRSA. Some of the newer MRSA selective media such as chromogenic
34
35 341 media for MRSA that use cefoxitin as the selective agent will not detect *mecA*-negative
36
37 342 BORSA isolates as we have observed in our own clinical laboratory. Thus these isolates
38
39 343 could be missed from CF respiratory specimens unless full antimicrobial susceptibilities
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41 344 were performed on all *S. aureus* isolates. Hence, CF patients could potentially be treated
42
43 345 with suboptimal antibiotics during a pulmonary exacerbation.

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45
46 346 There were several limitations to this study. It is unclear whether CF patients
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48 347 infected with BORSA isolates behave more like CF patients infected with MSSA or CF
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50 348 patients infected with MRSA Future studies assessing the clinical impact of BORSA in
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3 349 CF should compare BORSA, MRSA and MSSA colonized patients in a longitudinal
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5 350 fashion, controlling for potential confounding factors and include more extensive PFGE
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8 351 comparisons to determine the evolution of BORSA. In addition, the small number of
9
10 352 patients limited the number of variables that could be included in the logistic regression
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12 353 model of risk factors. This study was also not powered to detect differences in clinical
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15 354 outcomes between the groups. It was a single centre study and may not reflect findings in
16
17 355 other CF populations. Finally, in a quarter of patients with *mecA*-negative BORSA, the
18
19 356 mechanism of resistance to methicillin remains unknown.

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21
22 357 In conclusion, CF patients with *mecA*-positive MRSA most commonly acquire
23
24 358 their infection through person to person transmission whereas BORSA is likely
25
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27 359 preferentially selected out from endogenous MSSA in CF patients due to persistent
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29 360 antibiotic pressure. Prospective studies are needed to examine the prevalence, clinical
30
31 361 impact and appropriate management of BORSA in the CF population, as well as the
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33 362 underlying mechanism of resistance.

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6
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9
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11
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13
14 373 assistance in the data analysis.
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3 **Figure legends**
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5 **Figure 1.**
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8 A. PFGE of *SmaI* restriction fragments of representative MRSA isolates (lanes 2-6).
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10 Strains in lanes 3, 4 are indistinguishable and strains in lanes 2, 6 are closely related.
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12 Lanes 1,7: lambda ladder.
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15 B. Representative BORSA isolates (lanes 1-5) that are unrelated. Lane 6: lambda ladder.
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4 1 **Epidemiology of borderline oxacillin-resistant *Staphylococcus***
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7 2 ***aureus* in pediatric cystic fibrosis.**
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46 19 8963503, email address leahyt@tcd.ie
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49 20 **Keywords:** methicillin resistant *Staphylococcus aureus* (MRSA), *mecA*, pulsed-field gel
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51 21 electrophoresis.
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54 22 **Abbreviated title.** BORSA in pediatric cystic fibrosis
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24 Summary

25 A single-centre retrospective study was undertaken in children with cystic fibrosis (CF)
26 to evaluate 1) risk factors for acquisition; 2) molecular epidemiology; and 3) impact on
27 disease progression of borderline oxacillin-resistant *Staphylococcus aureus* (BORSA)
28 versus *mecA*-positive methicillin-resistant *Staphylococcus aureus* (MRSA). The study
29 comprised of 1) identification of all children with at least one respiratory specimen
30 positive for either BORSA or MRSA during the study period; 2) compilation of relevant
31 clinical and epidemiological data from 12-month period leading up to first positive
32 (index) culture; 3) microbiological and molecular characterisation of index isolates and 4)
33 measurement of subsequent clinical outcome. Thirty-eight children were identified with
34 at least one positive isolate; 4 were excluded due to insufficient clinical or laboratory
35 data. Eighteen children (53%) grew BORSA in their index culture. Children who
36 acquired BORSA only (n=16) were more likely to have had prior MSSA colonization
37 (p<0.0001). Usage of oral cephalexin (p<0.01) and inhaled tobramycin (p<0.03) prior to
38 index culture was significantly and independently associated with acquisition of BORSA.
39 The majority of BORSA isolates were hyper β -lactamase producers and susceptible to a
40 greater range of antibiotics. Strain relatedness was not evident within the BORSA group.
41 There was no difference in disease progression between the two groups. This is the first
42 study to demonstrate that a significant proportion of *S. aureus* isolates with methicillin
43 resistance in the CF population are BORSAs that lack *mecA*. Antibiotic pressure may
44 lead to the development of BORSA in CF patients. Prospective studies are needed to
45 assess its clinical impact.

46 Introduction

47
48 Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as an important
49 respiratory pathogen in the cystic fibrosis (CF) population. The US CF registry reported a
50 significant rise in MRSA infection among CF patients, from 2.1% in 1996 to 21.2% in
51 2007¹. MRSA infection has been associated with accelerated deterioration in pulmonary
52 function², increased hospitalization³, impaired growth, increased antibiotic usage⁴ and
53 increased mortality in CF patients⁵.

54 According to the Clinical Laboratory Standards Institute (CLSI), methicillin
55 resistance in *S. aureus* is defined as having an oxacillin minimum inhibitory
56 concentration (MIC) > 4 µg/ml⁶. The primary mechanism of resistance for MRSA is the
57 production of an altered penicillin binding protein (PBP2a) with reduced affinity for
58 methicillin that is encoded by the *mecA* gene⁷. We have identified a group of CF patients
59 infected with borderline resistant *S. aureus* (BORSA) isolates that are characterized by
60 oxacillin MICs at or just above the susceptibility breakpoint of 4 µg/ml and lack the
61 *mecA* gene⁷. The implications of isolating BORSA in a respiratory culture from CF
62 patients have not previously been explored. The aim of this study was therefore to
63 compare the risk factors for acquisition, the molecular epidemiology and subsequent
64 clinical impact on disease progression of BORSA infection in comparison with *mecA*-
65 positive MRSA infection in pediatric CF patients.

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67

68 **Materials and Methods**

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70 Study design and patient population

71 This was a single-centre retrospective comparative study of pediatric CF patients
72 whose care was primarily based at the CF clinic at the Hospital for Sick Children,
73 Toronto between 1992 and 2007. The clinic currently cares for approximately 280
74 children up to the age of eighteen. Respiratory samples (expectorated sputum or
75 oropharyngeal swabs) were sent from all children attending the clinic to the microbiology
76 laboratory for culture at every 3-month visit and during acute pulmonary exacerbations
77 (APEs). Patients with one or more respiratory specimens positive for *S. aureus* with
78 methicillin resistance (either BORSA or MRSA) were included in the analysis. Cases
79 with three or more specimens positive for BORSA or MRSA during the study period
80 were considered persistently colonized². The study was approved by the Hospital for
81 Sick Children's Research Ethics Board.

82

83 Data collection

84 Patients with BORSA or MRSA isolated from their respiratory specimens were
85 identified using the microbiology database, and cross-referenced with the CF clinical
86 database. Date of the first BORSA or MRSA-positive respiratory culture (index culture)
87 for each patient was identified. The medical and laboratory records for each patient were
88 examined using a standardized data collection form. Baseline patient characteristics were
89 assessed at the date of index culture, and risk factors for acquisition of BORSA or MRSA
90 infection were assessed over the twelve-month period prior to the index culture. Impact

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3 91 on clinical progression was assessed by examination of the time period from index
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5 92 culture until discharge from the clinic or until December 31st 2008, whichever date was
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8 93 later. Data collected included information on patients' demographics, pulmonary function
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10 94 tests (PFTs), respiratory cultures, hospital admissions, acute pulmonary exacerbations
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12 95 and antibiotic use. An acute pulmonary exacerbation was defined as a change in
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14 96 pulmonary status considered by the attending physician to require antibiotic treatment,
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16 97 either oral or intravenous. An "antibiotic day" was defined as one day for each antibiotic
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18 98 used, for example a 14-day course of two antibiotics was defined as 28 "antibiotic days".
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20 99 The purpose of this distinction was to capture more complex antibiotic regimens that
21
22 100 might be employed to treat MRSA infected patients. Patients were classified as being on
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24 101 oral cephalexin if they were prescribed cephalexin at the time of index culture of at least
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26 102 one month's duration (in all instances, this occurred in the absence of signs or symptoms
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28 103 of a pulmonary exacerbation). Patients were classified as being on inhaled tobramycin if
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30 104 they were prescribed inhaled tobramycin at the time of index culture of at least one
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32 105 month's duration (for previous *P. aeruginosa* infection). Patients were classified as being
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34 106 on oral ciprofloxacin if they were prescribed at least one course of ciprofloxacin (in all
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36 107 instances, this occurred in the presence of signs or symptoms of a pulmonary
37
38 108 exacerbation). Patients were categorized as co-colonized with other CF pathogens using
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40 109 previously defined criteria⁸. Only patients with at least 6 months of follow-up after the
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42 110 index culture were included in the analysis of outcome measures.
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3 114 Microbiology
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5 115 **Specimen Processing**
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8 116 Respiratory specimens from CF patients were screened for MRSA using media
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10 117 containing 2 µg/ml of oxacillin: an in-house mannitol salt agar (MRSA agar base,
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12 118 Acumedia, Lansing, MI, and 1% D-mannitol, Difco, Detroit, MI) supplemented with 1%
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14 119 thymidine and 2 µg/ml of oxacillin (both from Sigma, St. Louis, MO) was used from
15
16 120 1992 to 2001, and Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid, Nepean,
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18 121 Ontario, Canada) was used from 2002 onward. Plates were incubated at 35°C aerobically
19
20 122 and observed for the presence of growth at 24 hours and at 48 hours. Suspicious colonies
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22 123 were identified as *S. aureus* by Gram stain, catalase, slide coagulase, and tube coagulase
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24 124 tests.
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32 126 **Antimicrobial Susceptibility**
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34 127 Antimicrobial susceptibility of *S. aureus* isolates was tested by a combination of
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36 128 oxacillin 6µg/ml screen plate, disk diffusion^{6,9} and an automated system (Vitek, bio-
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38 129 Merieux, St. Laurent, Quebec, from 1992 to Nov 2002, and the BD Automated Phoenix
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41 130 System, BD Diagnostic Systems, Sparks, MD from Nov 2002 onward). Inducible
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43 131 resistance to clindamycin was detected by disk diffusion using the D-zone test^{6,9}. In
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45 132 addition, each strain was examined for the presence of PBP2a by latex agglutination
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47 133 (PBP2' test kit, Oxoid, Hants, UK). Organisms that were negative for PBP2a by latex
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49 134 agglutination but grew on the oxacillin screen plate were sent to the reference laboratory
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51 135 (Central Provincial Health Laboratory, Ontario) for minimum inhibitory concentration
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53 136 (MIC) by agar dilution and polymerase chain reaction (PCR) for the detection of the
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3 137 *mecA* gene. An isolate was deemed as BORSA if the oxacillin MIC was $\geq 4 \mu\text{g/ml}$,
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5 138 PBP2a was absent and the *mecA* PCR was negative⁷. An isolate was deemed as MRSA if
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8 139 the oxacillin MIC was $\geq 4\mu\text{g/ml}$, the latex agglutination for PBP2a was positive and/or the
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10 140 *mecA* PCR was positive⁶.
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142 **β -Lactamase Production**

143 All BORSAs were tested for the production of β -lactamase as a potential
144 mechanism of oxacillin resistance by disk diffusion with ampicillin (10 μg) and
145 amoxicillin-clavulanic (20 μg / 10 μg) disks as previously described (13). An isolate was
146 deemed as hyper β -lactamase producing if the ampicillin zone size was resistant and the
147 amoxicillin-clavulanic zone size was susceptible, suggesting borderline resistance was
148 secondary to large amounts of β -lactamase⁹(13).
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150 **Molecular Characterization**

151 The *mecA* and *nuc* gene were co-amplified with a multiplex real time PCR as
152 described previously¹⁰. The presence of Panton-Valentine leukocidin was examined by
153 amplification of the *lukF-PV* and *lukS-PV* genes using primer and protocols previously
154 described¹¹. Pulsed-field gel electrophoresis (PFGE) was conducted using *SmaI* as per
155 manufacturer protocol (Bio-rad Laboratories, Hercules, CA) on all newly identified
156 MRSA strains. Electrophoretically-generated DNA profiles were analyzed using the
157 criteria proposed by Tenover et al to determine strain relatedness¹².
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3 160 Statistical analysis
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5 161 Descriptive statistics, frequency distributions and percentages were calculated for
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8 162 the outcome variables and other covariates of interest. Normality assumption test was
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10 163 carried out for the continuous variables using the Kolmogorov-Smirnov test. Student's t-
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12 164 test or Kruskal-Wallis test (if normality assumption failed) was conducted for continuous
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14 165 variables within the two groups. The Chi-square or Fisher's exact test, as appropriate,
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16 166 was carried out for the dichotomous variables to assess the association with the two
17
18 167 groups. To analyze independent risk factors, a multivariate analysis using a logistic
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20 168 regression model was applied. A p-value of <0.05 was considered statistically significant.
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22 169 The statistical programme SAS (version 9.1; SAS Institute Inc., Cary, NC) was used.
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3 171 **Results**

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8 173 Patient population

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11 Between 1992 and 2007, 38 patients had at least one respiratory specimen
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13 positive for either BORSA or MRSA. Of these, three were excluded from our analysis
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15 because their care was not primarily based at our hospital, thus we had no clinical data
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17 for these patients. In addition, *mecA* PCR could not be performed on one isolate
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19 retrospectively therefore 34 patients were included in the study. Of these 34 patients, two
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21 patients were initially colonized with *mecA*- negative and subsequently *mecA*-positive
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23 strains and were excluded from the final statistical analysis as it was unclear how to
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25 categorize them in terms of patient characteristics and outcomes. Ultimately 16 patients
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27 with *mecA* positive MRSA and 16 with *mecA* negative BORSA on the index culture were
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29 compared for baseline characteristics. For the longitudinal assessment, 15 patients in each
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31 group were compared since one patient in each group did not have follow-up data for at
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33 least 6 months.
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42 187 Patient characteristics and risk factors for BORSA and MRSA

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44 188 The two groups were similar in terms of baseline characteristics (Table 1). There
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46 189 was no difference in pulmonary function or rate of decline of pulmonary function in the
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48 190 year prior to the index culture. In addition, there was no difference in the number of acute
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50 191 pulmonary exacerbations, hospital admissions or overall antibiotic use for the treatment
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52 192 of acute pulmonary exacerbations. A significantly greater proportion of BORSA infected
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54 193 patients were colonized with MSSA in the year prior (94% vs 25%, $p < 0.0001$). Likewise,
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3 194 a higher proportion of BORSA infected patients received oral antibiotics (cephalexin)
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5 195 (75% vs 25%, p=0.005).
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8 196 Mean duration of therapy among those patients on cephalexin was 337 days (range 30-
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10 197 365 days) in the BORSA group and 311 days (range 150-365 days) in the MRSA group;
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12 198 cephalexin treatment was instituted not for pulmonary exacerbation but as treatment for
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15 199 MSSA identified on routine sputum cultures. Furthermore, a significantly greater
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17 200 number of BORSA infected patients had received a course of oral ciprofloxacin (31% vs
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19 201 0%, p=0.043) or were on inhaled antibiotics (tobramycin) (88% vs 44%, p=0.009) in the
20
21 202 year prior to the index culture than patients with MRSA. No inter-group difference was
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23 203 detected in any of the other co-colonizing respiratory pathogens. Patients with BORSA
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25 204 strains had significantly more clinic visits in the year prior to first isolate (n=7.94) than
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27 205 patients with *mecA*-positive strains (n=4.69, p=0.049) although this was not a significant
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29 206 risk factor in the logistic regression model
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34 207 A logistic regression model was applied to determine the independence of
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36 208 epidemiological and clinical variables that were correlated with BORSA infection.
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38 209 Colonization with MSSA and use of oral cephalexin were significantly associated as 68%
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40 210 of patients with MSSA were treated with cephalexin and 81% of patients treated with
41
42 211 cephalexin were colonized with MSSA (p<0.05). The use of oral cephalexin (regression
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44 212 coefficient (β)=1.15, SD=0.47, p value=0.015) and inhaled tobramycin (regression
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46 213 coefficient (β)=1.16, SD=0.53, p value=0.028) in the year prior to index culture were
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48 214 significantly and independently associated with BORSA infection.
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55 216 Microbiological characteristics of BORSA and MRSA isolates
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3 217 The microbiological characteristics of the BORSA isolates are illustrated in Table
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5 218 2. Fourteen of the 16 isolates had oxacillin MICs at or just above the breakpoint (4 to 8
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8 219 $\mu\text{g/ml}$) but 2 isolates had MICs of 16 $\mu\text{g/ml}$. All of the BORSA isolates were negative for
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10 220 PBP2a by latex agglutination and were *mecA* negative by PCR. Twelve of the 16 BORSA
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12 221 isolates were hyper β -lactamase producers. This was shown by resistance to ampicillin by
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14 222 disk diffusion that was reverted to susceptible with the addition of clavulanic acid, a β -
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16 223 lactamase inhibitor. The remaining 4 isolates had ampicillin zone size that were
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18 224 unchanged by addition of clavulin by disk diffusion.
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22 225 In comparison to the MRSA isolates, a significantly greater proportion of the
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24 226 BORSA isolates were susceptible to both erythromycin (81% vs 13%, $p < 0.0001$) and
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26 227 clindamycin (94% vs 31%, $p < 0.001$). None of the BORSA isolates were D-test positive
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28 228 but 5 (45%) of the MRSA isolates were D-test positive ($p = 0.043$). All of the BORSA
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30 229 and MRSA isolates were susceptible to trimethoprim-sulphamethoxazole. None of the
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32 230 BORSA isolates tested produced PVL. Three of the 16 MRSA isolates were identified as
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34 231 USA300 epitypes, and produced PVL.
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41 232 Relatedness of BORSA and MRSA isolates

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43 234 Pulsed field gel electrophoresis (PFGE) revealed that none of the BORSA strains
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45 235 were related by the Tenover criteria¹³ (Figure 1). Within the MRSA group, we found
46
47 236 four separate instances of strain relatedness during the course of the study: a pair of
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49 237 siblings shared an indistinguishable strain, four patients shared possibly related strains,
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51 238 two patients shared a closely related strain with a third patient sharing a possibly related
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3 239 strain and, finally, three patients (including a pair of siblings) shared an indistinguishable
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5 240 strain.
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10 242 Clinical outcomes of patients

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12 243 To compare the clinical impact of BORSA versus MRSA infection on disease
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14 244 progression, we assessed 99.7 patient-years of follow-up after the index culture with a
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16 245 comparable mean duration of follow up of 3.37 years in the MRSA group and 3.27 years
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18 246 in the BORSA group ($p=0.507$) (Table 3). Patients infected with MRSA were more
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20 247 likely to have persistent infection at the end of follow up than patients with BORSA
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22 248 infection (80% vs 53%) but this was not statistically significant ($p=0.121$). The annual
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24 249 decline in percent predicted forced expiratory volume in 1 second (FEV_1), was greater
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26 250 among the MRSA infected patients (5.8%) than those infected with BORSA (2.8%)
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28 251 although this difference was not significant ($p = 0.77$). Neither was there a significant
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30 252 difference in the number of acute pulmonary exacerbations, hospital admissions, inpatient
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32 253 days or clinic visits. One patient in the MRSA group died, and another progressed to lung
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34 254 transplant. Three patients in the BORSA group had a lung transplant during the study
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36 255 period.
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3 257 **Discussion**
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8 259 This is the first study to show that a significant proportion (50%) of *S. aureus*
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10 260 isolates with methicillin resistance infecting CF patients are BORSA which lack the
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12 261 *mecA* gene, the main genetic determinant of methicillin resistance in *S. aureus*⁷. The
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14 262 present study demonstrates that antibiotic use in CF patients colonized with MSSA is a
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16 263 risk factor specifically for infection with BORSA. Previous studies have shown that
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18 264 ciprofloxacin and cephalosporin use is a risk factor for acquisition of MRSA in CF
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20 265 patients¹⁴ but, to our knowledge, no other study has examined the differential risk factors
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22 266 for BORSA compared to MRSA acquisition.
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27 267 Transmission of traditional MRSA between patients^{15,16}, within the community¹⁷
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29 268 and even within households¹⁸ has been well described in the CF population. Evidence of
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31 269 person to person transmission of *mecA*-positive MRSA was also present in this study
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33 270 with shared strains among CF patients and siblings. The mechanism of resistance is
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35 271 relevant to how CF patients acquire MRSA as the *mecA* gene is absent in its entirety from
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37 272 MSSA^{19,7}. It is believed to have originated from another staphylococcus species, such as
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39 273 *Staphylococcus sciuri*, and been transposed into MSSA²⁰. Although horizontal transfer
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41 274 of *mecA* between a *S. epidermidis* and MSSA isolate within the same patient has been
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43 275 described²¹, transmission of *mecA*-positive MRSA isolates between individuals is likely
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45 276 more common as there are a well defined number of *mecA*-positive MRSA clones
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47 277 worldwide²².
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53 278 Our data would suggest that CF patients may acquire *mecA*-negative BORSA
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55 279 isolates in a different way. In contrast to MRSA isolates, BORSA isolates had unique
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3 280 PFGE patterns and appeared not to be shared between patients in our study. CF patients
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5 281 colonized with MSSA and exposed to oral cephalexin or inhaled tobramycin (which can
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7 282 act synergistically with β -lactam antibiotics against *S. aureus*) were at significantly
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9 283 increased risk of acquiring BORSA infection. This suggests that BORSA isolates may
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11 284 arise from endogenous MSSA isolates that develop other mechanisms of resistance to
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13 285 methicillin in response to antibiotic pressure. Previous studies have shown that *S. aureus*
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15 286 strains with borderline susceptibility to oxacillin have a significant drop in the penicillin
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17 287 MICs to the susceptible range with the addition of a β -lactamase inhibitor²³. This
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19 288 predicts the response of oxacillin MICs to the addition of a β -lactamase inhibitor and
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21 289 suggests that a large amount of β -lactamase production is the mechanism of resistance to
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23 290 oxacillin in the majority of BORSA, as observed in 12 of 16 isolates in our study.
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25 291 However, in the 4 remaining BORSA strains where the ampicillin zone size did not
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27 292 change significantly with the addition of clavulanic acid, the mechanism of methicillin
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29 293 resistance is unknown. In the aforementioned study, the authors described *S. aureus*
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31 294 strains with intermediate MICs to oxacillin that remained the same in the presence of
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33 295 clavulanic acid. The authors suggested that oxacillin resistance was due to factors other
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35 296 than β -lactamase inactivation²³. It is possible that it is due to a modification of “normal”
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37 297 penicillin-binding proteins (PBPs) such as PBP 1, PBP 2 and PBP 4 as was previously
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39 298 shown in clinical BORSA isolates by Tomasz et al²⁴. Exposure of susceptible
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41 299 staphylococci to increasing concentrations of β -lactam antibiotics in the laboratory is
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43 300 known to cause PBP alterations associated with increased MICs²⁵. In this study,
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45 301 persistent antibiotic pressure may have similarly modified PBPs in endogenous MSSA
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47 302 isolates colonizing CF patients and resulting in the development of *mecA*-negative, non-
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3 303 hyper β lactamase producing BORSA isolates. Thus, in the prevention of the emergence
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5 304 of BORSA in CF patients, other infection control measures, such as patient segregation,
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8 305 may not be as important as the judicious use of antibiotics.
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10 306 In our study, the majority of patients treated with oral cephalexin were colonized
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12 307 with MSSA. Although there were no signs and symptoms of a pulmonary exacerbation at
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14 308 the time, we are limited in a retrospective study to the information that is recorded in the
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16 309 medical charts. Their antibiotic treatment was thus likely driven by their MSSA positive
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18 310 respiratory cultures and/or more subtle symptoms. Primary prophylaxis for *S. aureus* (the
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20 311 treatment of all newly diagnosed CF patients with antistaphylococcal agents irrespective
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22 312 of respiratory culture results) is not routinely practiced in North America due to concerns
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24 313 of *P. aeruginosa* acquisition^{26,27}. A systematic review of prophylactic antibiotics in
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26 314 cystic fibrosis, however, suggests that *P. aeruginosa* acquisition in this context is
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28 315 associated less frequently with narrow spectrum anti-staphylococcal antibiotics such as
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30 316 flucloxacillin compared with broader spectrum anti-staphylococcal antibiotics such as
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32 317 cephalexin²⁸. In our study, although patients treated with oral cephalexin were not
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34 318 receiving prophylaxis against *S. aureus*, they were receiving prolonged courses of
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36 319 cephalexin for MSSA detected on routine culture of the respiratory tract. This prolonged
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38 320 cephalexin use was identified as an independent risk factor for the acquisition of BORSA
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40 321 isolates and occurred recently, primarily in the last 5 years of the study.
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48 322 The clinical significance of BORSA infection in CF patients is unknown. Our
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50 323 study lacked a sufficient sample size and is not powered to clearly delineate the clinical
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52 324 impact of BORSA infection. Previous studies have reported accelerated deterioration in
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54 325 pulmonary function², increased hospitalization³, impaired growth, increased antibiotic
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3 326 usage⁴ and increased mortality⁵ in CF patients infected with MRSA. However, these
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5 327 studies are limited by a lack of information on the laboratory methodology of identifying
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8 328 MRSA and thus it is not known to what degree these effects are attributable to BORSA or
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10 329 MRSA.

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12 330 Detection of BORSA isolates may also influence the choice of antibiotics in the
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14 331 treatment of these infections in the setting of a pulmonary exacerbation. In our study,
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16 332 BORSA isolates were more susceptible to erythromycin and clindamycin than the MRSA
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18 333 isolates. This finding is not surprising as the *mecA* gene is located on a mobile genetic
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20 334 element (the Staphylococcal Cassette Chromosome *mec*: SCC*mec*) which can contain
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22 335 additional drug resistance genes²². Although earlier studies suggested that infections due
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24 336 to *mecA* negative BORSA isolates could be treated with β -lactam antibiotics^{29,30}, more
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26 337 recent cases report failure of cloxacillin and even vancomycin in the treatment of
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28 338 endocarditis caused by BORSA, depending on the mechanism of methicillin resistance
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30 339^{31,32}. This has implications for how respiratory specimens from CF patients should be
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32 340 screened for MRSA. Some of the newer MRSA selective media such as chromogenic
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34 341 media for MRSA that use cefoxitin as the selective agent will not detect *mecA*-negative
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36 342 BORSA isolates as we have observed in our own clinical laboratory. Thus these isolates
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38 343 could be missed from CF respiratory specimens unless full antimicrobial susceptibilities
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40 344 were performed on all *S. aureus* isolates. Hence, CF patients could potentially be treated
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42 345 with suboptimal antibiotics during a pulmonary exacerbation.

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44 346 There were several limitations to this study. It is unclear whether CF patients
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46 347 infected with BORSA isolates behave more like CF patients infected with MSSA or CF
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48 348 patients infected with MRSA Future studies assessing the clinical impact of BORSA in
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3 349 CF should compare BORSA, MRSA and MSSA colonized patients in a longitudinal
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5 350 fashion, controlling for potential confounding factors and include more extensive PFGE
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8 351 comparisons to determine the evolution of BORSA. In addition, the small number of
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10 352 patients limited the number of variables that could be included in the logistic regression
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12 353 model of risk factors. This study was also not powered to detect differences in clinical
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15 354 outcomes between the groups. It was a single centre study and may not reflect findings in
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17 355 other CF populations. Finally, in a quarter of patients with *mecA*-negative BORSA, the
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19 356 mechanism of resistance to methicillin remains unknown.

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22 357 In conclusion, CF patients with *mecA*-positive MRSA most commonly acquire
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24 358 their infection through person to person transmission whereas BORSA is likely
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27 359 preferentially selected out from endogenous MSSA in CF patients due to persistent
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29 360 antibiotic pressure. Prospective studies are needed to examine the prevalence, clinical
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31 361 impact and appropriate management of BORSA in the CF population, as well as the
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33 362 underlying mechanism of resistance.
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9
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14 373 assistance in the data analysis.
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3 **Figure legends**
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5 **Figure 1.**
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8 A. PFGE of *Sma*I restriction fragments of representative MRSA isolates (lanes 2-6).
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10 Strains in lanes 3, 4 are indistinguishable and strains in lanes 2, 6 are closely related.
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12 Lanes 1,7: lambda ladder.
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15 B. Representative BORSA isolates (lanes 1-5) that are unrelated. Lane 6: lambda ladder.
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Table 1. Characteristics of patients with MRSA and BORSA

	MRSA (n=16)	BORSA (n=16)	P-value
Patient characteristics at index culture			
Mean age (range) in yr	10.4 (1.8-17.5)	13.1 (8.5-17.3)	0.078
No. (%) male	10 (63)	8 (50)	0.476
Mean BMI z-score	-0.18	-0.40	>0.5
No. (%) homozygous DF508	7 (44)	7 (44)	>0.5
No. (%) pancreatic insufficient	15 (94)	14 (88)	>0.5
No. (%) CF related diabetes	1 (6)	2 (13)	>0.5
Mean % predicted FEV ₁ (range)	68.5 (41-99)	69.4 (30-113)	>0.5
Respiratory flora in 12-month period prior to index culture			
No. (%) MSSA colonized	4 (25)	15 (94)	<0.0001
No. (%) <i>P. aeruginosa</i> colonized	3 (19)	6 (38)	0.433
No. (%) <i>A. fumigates</i> colonized	1 (6)	5 (31)	0.171
No. (%) <i>H. influenza</i> colonized	1 (6)	1 (6)	>0.5
No. (%) <i>B. cepacia</i> colonized	1 (6)	0 (0)	>0.5
No. (%) <i>S. maltophilia</i> colonized	1 (6)	1 (6)	>0.5
Antibiotic usage in 12-month period prior to index culture			
No. (%) of patients on oral cephalexin	4 (25)	12 (75)	0.005
No. (%) of patients on inhaled tobramycin	7 (44)	14 (88)	0.009
No. (%) of patients on oral ciprofloxacin	0(0)	5(31)	0.043
Disease activity during 12-month period prior to index culture			
Mean change (range) in % predicted FEV ₁	-2.5 (-11.6 to +10.7)	-3.8 (-28.9 to +10.2)	>0.5
Mean no. (range) of exacerbations	0.8 (0-4)	1.4 (0-8)	>0.5
Mean no. (range) of hospital admissions	0.4 (0-4)	0.7 (0-6)	>0.5
Mean no. (range) of inpatient days	3.4 (0-37)	7.6 (0-61)	>0.5
Mean no. (range) of IV antibiotic days	6.1 (0-70)	14.2 (0-130)	>0.5
Mean no. (range) of clinic visits	4.7 (3-10)	7.9 (3-35)	0.049

Abbreviations: BMI – body mass index, MSSA- methicillin sensitive *Staphylococcus aureus*, FEV₁-

Forced expiratory volume in 1 second.

Table 1. Characteristics of patients with MRSA and BORSA

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No. (%) pancreatic insufficient	15 (94)	14 (88)	>0.5
No. (%) CF related diabetes	1 (6)	2 (13)	>0.5
Mean % predicted FEV ₁ (range)	68.5 (41-99)	69.4 (30-113)	>0.5
Respiratory flora in 12-month period prior to index culture			
No. (%) MSSA colonized	4 (25)	15 (94)	<0.0001
No. (%) <i>P. aeruginosa</i> colonized	3 (19)	6 (38)	0.433
No. (%) <i>A. fumigates</i> colonized	1 (6)	5 (31)	0.171
No. (%) <i>H. influenza</i> colonized	1 (6)	1 (6)	>0.5
No. (%) <i>B. cepacia</i> colonized	1 (6)	0 (0)	>0.5
No. (%) <i>S. maltophilia</i> colonized	1 (6)	1 (6)	>0.5
Antibiotic usage in 12-month period prior to index culture			
No. (%) of patients on oral cephalexin	4 (25)	12 (75)	0.005
No. (%) of patients on inhaled tobramycin	7 (44)	14 (88)	0.009
No. (%) of patients on oral ciprofloxacin	0(0)	5(31)	0.043
Disease activity during 12-month period prior to index culture			
Mean change (range) in % predicted FEV ₁	-2.5 (-11.6 to +10.7)	-3.8 (-28.9 to +10.2)	>0.5
Mean no. (range) of exacerbations	0.8 (0-4)	1.4 (0-8)	>0.5
Mean no. (range) of hospital admissions	0.4 (0-4)	0.7 (0-6)	>0.5
Mean no. (range) of inpatient days	3.4 (0-37)	7.6 (0-61)	>0.5
Mean no. (range) of IV antibiotic days	6.1 (0-70)	14.2 (0-130)	>0.5
Mean no. (range) of clinic visits	4.7 (3-10)	7.9 (3-35)	0.049

Abbreviations: BMI – body mass index, MSSA- methicillin sensitive *Staphylococcus aureus*, FEV₁-

Forced expiratory volume in 1 second.

Table 2. Microbiological characteristics of BORSA isolates

Isolate	Oxacillin MIC (µg/ml)	PBP2a latex agglutination	<i>mecA</i> PCR	AMP/AMC zone size (mm)†	Hyper β-lactamase production	ERY	CLI	SXT
SA 002	8	neg	neg	13/23	Yes	S	S	S
SA 003	16	neg	neg	12/26	Yes	I	S*	S
SA 004	4	neg	neg	38/40	No	S	S	S
SA 006	8	neg	neg	35/36	No	S	S	S
SA 011	8	neg	neg	18/29	Yes	S	S	S
SA 016	4	neg	neg	15/26	Yes	S	S	S
SA 018	8	neg	neg	14/28	Yes	S	S	S
SA 020	4	neg	neg	17/28	Yes	S	S	S
SA 021	4	neg	neg	16/26	Yes	S	S	S
SA 022	8	neg	neg	30/29	No	S	S	S
SA 023	8	neg	neg	16/21	Yes	R	S*	S
SA 029	16	neg	neg	10/27	Yes	S	S	S
SA 034	8	neg	neg	12/21	Yes	S	S	S
SA 035	4	neg	neg	29/31	No	S	S	S
SA 036	4	neg	neg	16/23	Yes	S	S	S
SA 038	8	neg	neg	11/24	Yes	R	S*	S

† Ampicillin (AMP) susceptible zone size ≥ 29 mm, resistant ≤ 28 mm

Amoxicillin clavulanic acid (AMC) susceptible zone size ≥ 20 mm, resistant ≤ 19 mm⁹

* D-test negative, AMP-ampicillin, AMC-amoxicillin clavulanic acid

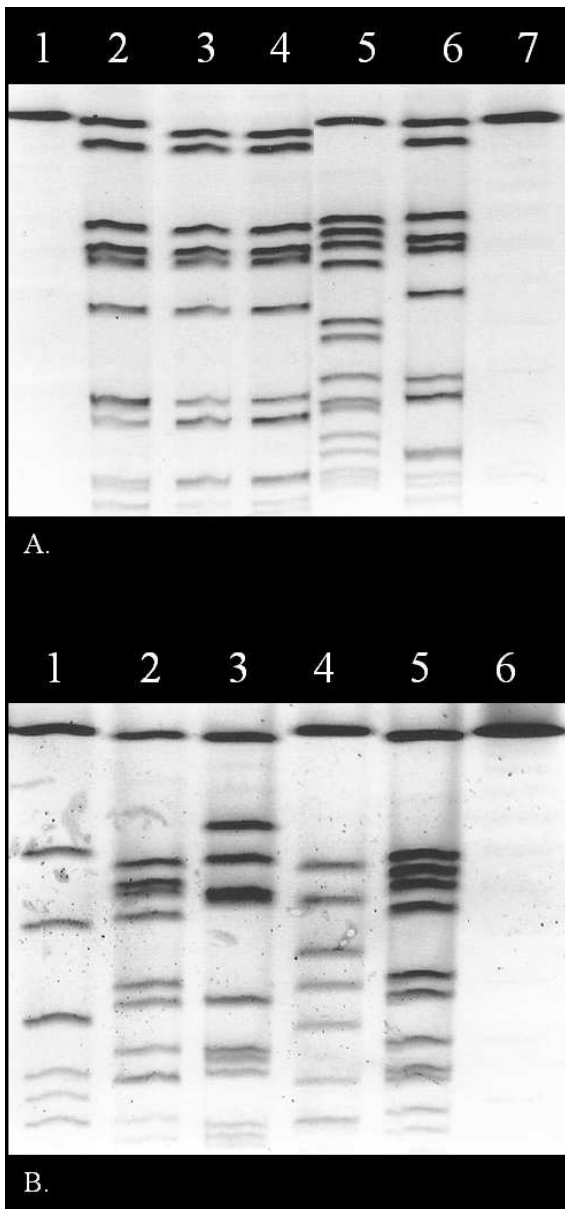
ERY-erythromycin, CLI-clindamycin, SXT-trimethoprim-sulphamethoxazole

S-susceptible I-intermediate R-resistant

Table 3. Clinical outcomes of patients with MRSA and BORSA

	MRSA	BORSA	P-
Clinical outcomes	(n = 15)	(n = 15)	value
Mean duration (range) of follow up in years	3.37 (0.76-14.8)	3.27 (0.66-5.57)	0.507
Mean annual change (range) in % predicted FEV1	-5.8 (-33.1 to +3.2)	-2.8 (-10.2 to +7.7)	0.770
Mean no. (range) of exacerbations per year	1.8 (0-5.3)	2.4 (0.3-7.6)	0.443
Mean no. (range) of hospital admissions per year	0.9 (0-6.6)	1.3 (0-4.7)	0.242
Mean no. (range) of inpatient days per year	11.5 (0-109.2)	16.1 (0-91.7)	0.226
Mean no. (range) of IV antibiotic days per year	26.1 (0-224.1)	33.3 (0-200.6)	0.361
Mean no. (range) of clinic visits per year	7.7 (3.7-37.4)	11.7 (2.8-80.9)	0.272
No. (%) of patients with persistent infection	12 (80)	8 (53.3)	0.121

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83x176mm (150 x 150 DPI)