The Predominant Variant of the Brazilian Epidemic Clonal Complex of Methicillin-Resistant Staphylococcus aureus Has an Enhanced Ability to Produce Biofilm and to Adhere to and Invade Airway Epithelial Cells

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Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a therapeutic problem. In the present study, the molecular characterization by pulsed-field gel electrophoresis of MRSA isolates collected from a university hospital revealed that the predominant variant of the Brazilian epidemic clonal complex (BECC) was responsible for the increase in the incidence of MRSA strains, which reached 28% in 1998. It was verified that this predominant variant of the BECC displayed an enhanced ability to produce biofilm on inert polystyrene surfaces and to adhere to and invade epithelial airway cells. These results indicate that MRSA strains belonging to the BECC have evolved advantageous properties that might play a role in their predominance as international nosocomial pathogens.

Methicillin-resistant Staphylococcus aureus (MRSA) is the leading cause of hospital infections worldwide [1–3]. At the end of the 1980s and the beginning of the 1990s, the medical community was overwhelmed by a dramatic increase in infections caused by multidrug-resistant S. aureus in nosocomial settings. This increase was associated with an international dissemination of well-defined clonal lineages (epidemic MRSA) [1, 3]. Five major lineages (the so-called Iberian, Brazilian, Hungarian, New York/Japan, and pediatric) of pandemic MRSA clones have been defined [4, 5]. In Brazil, the Brazilian epidemic clonal complex (BECC) of MRSA has disseminated from the north to the south [6–8]. BECC isolates have also been detected in other countries, including Argentina, Uruguay, Paraguay, Chile, Portugal, Italy, and the Czech Republic [9–13]. The mechanisms involved in the selection of pandemic MRSA clones have not been clarified thus far. Nevertheless, the ability of S. aureus to establish infections seems to be dependent on the expression of virulence factors that enable the bacterial colonization of mucosal or skin surfaces.

In the present study, we demonstrate that the predominant isolate of the BECC displays an enhanced in vitro ability to produce biofilm and to adhere to and invade the epithelial bronchial cell line 16HBE14o-. This finding strongly suggests that this isolate displays selective properties that might have contributed to the spread and predominance of the BECC of MRSA as a global nosocomial pathogen.
Table 1. Resistance profiles and pulsed-field gel electrophoresis (PFGE) patterns of the methicillin-resistant *Staphylococcus aureus* isolates from João Barros Barreto University Hospital.

<table>
<thead>
<tr>
<th>Disease (no. of isolates)</th>
<th>Drug resistance profile</th>
<th>PFGE pattern (no. of isolates)(^a)</th>
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<tbody>
<tr>
<td>Pn (1), BI (1)</td>
<td>CHL, CIP, CLI, ERY, GEN, OXA, PEN, RIF, SXT</td>
<td>A(_1) (1), non-A(_1) (1)</td>
</tr>
<tr>
<td>Pn (1), SI (1), OL (1), BI (1)</td>
<td>CIP, CLI, ERY, GEN, OXA, PEN, RIF, SXT, TET</td>
<td>A(<em>1) (3), D(</em>{2}) (1)</td>
</tr>
<tr>
<td>Pn (1), BI (4)</td>
<td>CHL, CIP, CLI, ERY, GEN, OXA, PEN, SXT, TET</td>
<td>A(_1) (3), non-A(_1) (2)</td>
</tr>
<tr>
<td>Pn (13), Pe (3), BI (44), SkI (28), SI (17), UI (12)</td>
<td>CHL, CIP, CLI, ERY, GEN, OXA, PEN, RIF, SXT, TET</td>
<td>A(_1) (66), non-A(_1) (39), B(_2) (1), B(_3) (1), B(_4) (1), C(_5) (1), C(_6) (1), C(_7) (1), C(_8) (1), D(_1) (1), D(<em>2) (2), E(</em>{1}) (1), F(_1) (1), G (2), H (1)</td>
</tr>
<tr>
<td>BI (1)</td>
<td>CHL, CLI, ERY, GEN, OXA, PEN, SXT, TET</td>
<td>Non-A(_1) (1)</td>
</tr>
<tr>
<td>BI (2), SkI (1)</td>
<td>CIP, CLI, ERY, GEN, OXA, PEN, RIF, SXT</td>
<td>A(_1) (3)</td>
</tr>
<tr>
<td>SkI (1)</td>
<td>CHL, CLI, ERY, GEN, OXA, PEN, RIF, TET</td>
<td>Non-A(_1) (1)</td>
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<tr>
<td>UI (1)</td>
<td>CHL, CIP, CLI, ERY, OXA, PEN, RIF, SXT, TET</td>
<td>C(_{10}) (1)</td>
</tr>
</tbody>
</table>

**NOTE.** BI, blood infection; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; OL, oral infection; OXA, oxacillin; Pe, peritonitis; PEN, penicillin; Pn, pneumonia; RIF, rifampin; SI, surgical infection; SkI, skin infection; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; UI, urinary tract infection.

\(^a\) The PFGE pattern A\(_1\) was identical to that of Brazilian epidemic clonal complex (BECC) strain HU25 [9]. Non-A\(_1\) (BECC subtypes A\(_2\) to A\(_{21}\)), isolates differing by 1–3 bands from pattern A\(_1\), B\(_1\), C\(_1\), D\(_1\), E\(_1\), and F\(_1\), isolates differing by 4–6 bands from pattern A\(_1\), that were classified as another type but are possibly related to the BECC; G and H, isolates differing by >7 bands from pattern A\(_1\) [5].
as described elsewhere [14]. S. aureus strain ATTC 25923 was used as a control.

**Molecular characterization.** Pulsed-field gel electrophoresis (PFGE) of Smal-digested DNA was performed on a CHEF-DR III (Bio-Rad) as described elsewhere [15]. The criteria used for interpreting the PFGE results have been described elsewhere [5]. The analyses of the Clal/meca and Clal/Tn.554 polymorphisms were performed as described elsewhere [16].

**Biofilm.** Biofilm assays were performed using trypticase soy broth (TSB) (Difco Laboratories) supplemented with 1% (wt/vol) glucose. Overnight cultures (diluted 1:100) were dispensed into sterile 96-well flat-bottom tissue culture plates (Nunclon; Nunc A/S). The other procedures were performed as described elsewhere [17], except that the optical density of the culture (ODg) and of the stained biofilm (ODb) were measured at 570 nm [18]. S. epidermidis strain 70D and S. pyogenes strain 75194 were used as positive and negative controls, respectively. Biofilm production was classified on the basis of the results obtained for the negative control. The biofilm unit (BU) was calculated using the following formula: ODb/ODg. The calculated BU for S. pyogenes was 0.115. We defined S. aureus isolates with BU ≤0.230 as nonproducers, those with BU >0.230 and ≤0.460 as weak producers, those with BU >0.460 and ≤0.920 as moderate producers, and those with BU >0.920 as strong producers. Because the production of biofilm is subject to phase variation, the test was performed in quadruplicate. When an isolate was negative for biofilm production, the test was repeated for up to 3 additional independent experiments. The highest BU value obtained for each isolate tested was used for the statistical calculations.

**Adherence.** The bronchial epithelial cell line 16HBE14o− [19] was provided by Dr. Maria Cristina Plotkowski (Universidade do Estado do Rio de Janeiro, Brazil). Cell monolayers were prepared on a glass coverslip (Clay Adams; Becton Dickinson) and placed in a 24-well flat-bottom plate (Gibco BRL) with M-199 medium containing 30% (wt/vol) HEPES (Gibco BRL), 2.5 mg/L fungizone, 50 mg/L gentamicin, and 10% (vol/vol) fetal calf serum. Cells were incubated for 2–3 days at 37°C in 5% CO2. Bacteria were grown up to OD610 0.4 (log phase) in TSB containing 10 μCi of [methyl-3H]thymidine (Amersham Pharmacia Biotech). Aliquots of 200 μL of the culture were laid onto the monolayer. After 1 h of incubation at 37°C, the coverslip was washed twice and was transferred to the counting scintillant (Amersham Pharmacia Biotech), and the radioactivity was measured. Three determinations were made for each isolate tested.

**Invasion assay.** Bacterial invasion was investigated using the 16HBE14o− cell line. The monolayer was prepared in 24-well flat-bottom plates. A volume of 500 μL of bacterial inoculum at log phase (OD610 0.4) or stationary phase (OD610 1.8) was added to the monolayers. After 3.5 h of incubation, monolayers were washed twice in M-199 medium and were

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**Figure 1.** Genetic diversity as determined by pulsed-field gel electrophoresis (PFGE). A, Distribution of the clonality of the methicillin-resistant Staphylococcus aureus (MRSA) population isolated from 1995 to 1998 in the João Barros Barreto University Hospital, Belém, Brazil. B, PFGE patterns of the isolates analyzed: λ-ladder molecular size marker (lanes 1 and 14), MRSA clones rarely detected in hospitals (sporadic clones; lanes 2, 3, and 4), isolates belonging to the Brazilian epidemic clonal complex (BECC; lanes 5 and 6), and isolates displaying the A1 PFGE pattern that is predominant among BECC isolates (BECC A1; lanes 7–13).

**MATERIALS AND METHODS**

**Bacterial isolates.** A total of 134 MRSA isolates were obtained from different clinical specimens from infected patients and wards in the João Barros Barreto University Hospital (Belém, Brazil) during 1995–1998. To study bacterial adherence to inert surfaces, a total of 200 S. aureus isolates were analyzed, including 150 MRSA and 50 methicillin-susceptible S. aureus (MSSA) isolates obtained during 1996–2001. Of the 150 MRSA isolates, 38 were from João Barros Barreto University Hospital, and the others were from hospitals located in different regions of Brazil or Argentina (100) and in the United States (12). The MSSA isolates studied were all associated with infections in patients in Brazilian hospitals. Seventeen MSSA isolates, 5 MRSA clones rarely detected in hospitals (sporadic MRSA clones), and 10 MRSA isolates belonging to the predominant subclone of the BECC were chosen at random for use in the adhesion and invasion experiments.

**Susceptibility test.** The disk diffusion test was performed using the Kirby-Bauer method as described elsewhere [19]. The analyses of the Clal/meca and Clal/Tn.554 polymorphisms were performed as described elsewhere [5]. The criteria used for interpreting the PFGE results have been described elsewhere [15]. The analyses of the Clal/meca and Clal/Tn.554 polymorphisms were performed as described elsewhere [16].
incubated with 10 μg/mL lysostaphin (specific activity, 500 U/mg; Sigma) for 20 min at 37°C to lyse noninvasive bacteria. Monolayers were washed again, incubated for 5 min with 0.25% (wt/vol) trypsin (specific activity, 11,000 U/mg; Sigma), and lysed during a 5-min incubation with 0.025% (vol/vol) Triton X-100 (Sigma). The number of colony-forming units per milliliter was determined in trypticase soy agar, and the percentage of bacterial invasion was calculated as described elsewhere [20].

The isolates studied were grouped on the basis of the percentage of bacteria that invaded cells as being highly invasive (≥10% of the bacteria invaded cells), invasive (1% to <10% of the bacteria invaded cells), poorly invasive (>0.001% to <1% of the bacteria invaded cells), or noninvasive (≤0.001% of the bacteria invaded cells). Noninvasive isolates were defined on the basis of the amount of lysis promoted by lysostaphin.

Electron microscopy. After 1 h of contact with bacteria, ep-
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Figure 3. Adherence and invasion of human bronchial cell line 16HBE14o by methicillin-resistant Staphylococcus aureus (MRSA) isolates. A, Percentage of adherence. Percentage of adherence was determined after 1 h of contact between the [methyl-3H]thymidine–labeled bacteria and the epithelial cell monolayer. B, Percentage of invasion. Percentage of invasion was determined after 3 h of contact between the bacteria and the monolayer. The internalized bacteria were counted (in colony-forming units per milliliter) after lysis of the epithelial cells. C, Percentage of invasion and optical density in different growth phases of isolate GV91. BECC, isolates belonging to the Brazilian epidemic clonal complex; BECC A1, isolates displaying the A1 pulsed-field gel electrophoresis pattern that is predominant among BECC isolates; MSSA, methicillin-susceptible S. aureus; sporadic clones, MRSA clones rarely detected in hospitals.

Effects of soluble fibronectin (Fn) and RGD peptide on bacterial invasion. The bacterial Fn-binding motifs were blocked by adding 0.1 μg/mL human plasma Fn (Gibco) to the cultures of the BECC isolates GV69 and GV91. When the OD610 reached 0.4, bacterial cells were washed twice with M-199 medium and added to the epithelial cell monolayer. Bacterial invasion was measured as described above.

To verify the involvement of RGD-dependent integrins on invasion by BECC isolates, the epithelial cells were treated with a 1 μg/mL synthetic RGD peptide (Gly-Arg-Gly-Asp-Thr-Pro; Gibco). The monolayer was washed twice with M-199 medium, and bacterial invasion was measured as described above. The concentrations of Fn and RGD peptide used were chosen on the basis of the results of previous studies [21, 22].

Effect of environmental conditions. To determine whether bacterial entry into airway cells could be modulated by environmental conditions, 0.9% (wt/vol) dextrose, 15 μg/mL MgSO4, or 7.5% (wt/vol) NaCl were added to the bacterial culture of the BECC isolates GV69 and GV91. To study the effect of pH, bacteria were grown in liquid medium containing 0.02 mol/L sodium acetate buffer (pH 5.0). The effect of temperature variation on bacterial invasion was also evaluated after bacteria were grown at 30°C and 40°C. Bacterial invasion was measured as described above.

Adherence to solid-phase Fn. To study the bacterial adherence to Fn, sterile glass coverslips were covered with 50 μg/mL human plasma Fn. The coverslips were incubated for 1 h at 37°C, left for 18 h at 4°C, washed 4 times with PBS, and covered with a solution of 0.1% (vol/vol) bovine serum albumin [23]. After 1 h of incubation at 37°C, the coverslips were washed twice with PBS. For inoculum preparation, a culture of the BECC isolate GV91 was incubated with 10 μCi of [methyl-3H]thymidine and incubated at 37°C until it reached an OD610 of 0.4 (log phase). In another experiment, [methyl-3H]thymidine thymidine was added when the bacterial culture achieved an OD610 of 1.0 (stationary phase), and the incubation lasted for 3 h. The cultures were laid on the Fn-coated coverslips. After 1 h of incubation at 37°C, the coverslips were washed twice with PBS. For inoculum preparation, a culture of the BECC isolate GV91 was incubated with 10 μCi of [methyl-3H]thymidine and incubated at 37°C until it reached an OD610 of 0.4 (log phase). In another experiment, [methyl-3H]thymidine thymidine was added when the bacterial culture achieved an OD610 of 1.0 (stationary phase), and the incubation lasted for 3 h. The cultures were laid on the Fn-coated coverslips. After 1 h of incubation at 37°C, the coverslips were washed twice with PBS and transferred to the counting scintillant, and the radioactivity was measured. The agr+ strain RN6911 (that has an increased expression of Fn-binding proteins) [24] and the isogenic agr− strain RN6390B (gift from Dr. Richard Novick, New York University, New York) were also used in these experiments.
Figure 4. Electron micrographs of the in vitro invasion of Staphylococcus aureus isolate GV91 (displaying the A1 pulsed-field gel electrophoresis pattern that is predominant among the Brazilian epidemic clonal complex) in the human bronchial cell line 16HBE14o− after 1 h of contact with the monolayer. A, Intact microvilli of a noninfected airway cell. B, Adhesion of bacterial hairlike structures to the apical surface of the microvilli. C, Collapse of microvilli characterized by a shortening and loss of these structures. D, Invasion by isolate GV91. Note that most dividing cells remain inside membrane vacuoles. Scale bar, 0.5 μm.

**Statistical calculations.** The test of hypotheses on 2 population means (unpaired data) was used to compare the mean BU. In the invasion and adhesion experiments, analysis of variance was applied for comparison of the different groups. Student’s t test was used for the invasion experiments on bacterial or cellular inhibitors and for the analyses of Fn-bound activity. P < .05 was considered to be statistically significant [25].

**RESULTS**

The 134 MRSA hospital isolates were all susceptible to vancomycin. However, high-level resistance was verified for the other drugs tested (table 1). Of the 134 MRSA hospital isolates analyzed, 76 (57%) had PFGE patterns (A1) that were indistinguishable from that displayed by strain HU25 [6], whose pattern predominates among the isolates belonging to the BECC (figure 1A–B). Forty (30%) isolates differed from pattern A1 by only 1–3 PFGE bands (patterns A1 to A13). Additionally, 15 (11%) isolates differed by 4–6 bands. Although they are possibly related to the BECC, these MRSA isolates were grouped into different types. Only 3 (2%) of the MRSA hospital isolates differed by ≥7 bands, and they were classified as unrelated clones G and H (table 1).

All isolates classified as belonging to the BECC displayed the Clal/mecA and Clal/Tn554 types III and B polymorphisms, respectively (data not shown), as was reported elsewhere for the BECC [6]. It is remarkable that the percentage of isolates displaying the A1 PFGE pattern (BECC predominant variant) in this hospital increased from 38% (1995) to 53% (1996) to 65% (1997) to 79% (1998), whereas the percentage of non-A1 BECC subclones and non-BECC isolates decreased (figure 1A). In 1994, when MRSA outbreaks began, the incidence of methicillin resistance among the S. aureus isolates in João Barros Barreto University Hospital was 7.2%. In 1995, when the present study was initiated, the incidence had increased to 15%. In subsequent years (1996–1998), the incidence varied from 26% to 28%. Data on the occurrence of MRSA in previous years were not recorded.

The mean BU for BECC A1 isolates was 2.09, which was
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Figure 5. Invasion by Staphylococcus aureus isolates GV69 and GV91 (displaying the A1 pulsed-field gel electrophoresis pattern that is predominant among the Brazilian epidemic clonal complex) in epithelial airway cells under different conditions. A, Bacterial cultures were treated with either nothing (control), 0.5 mg/mL protease, or 0.1 mg/mL fibronectin (Fn) before contact with epithelial cell monolayers, or epithelial cell monolayers were formed in supplemented M-199 medium (Materials and Methods) containing 1 mg/mL RGD peptide (Gly-Arg-Gly-Asp-Thr-Pro) before culture. B, Effect of environmental conditions on invasion. For this experiment, bacteria were grown in the conditions indicated before contact with the epithelial cell monolayers.

Figure 6. Fibronectin (Fn)–binding activity of Staphylococcus aureus strain RN6390B (agr+), strain RN6911 (an isogenic agr/H11002 mutant), and isolate GV91 (a predominant isolate of the Brazilian epidemic clonal complex). Binding activity was determined at log phase (OD610, 0.4) and stationary phase (OD610, 1.8) using solid-phase Fn.

significantly higher than that for MSSA isolates (BU, 0.50; P < .001), sporadic MRSA clones (BU, 0.72; P < .001), and other BECC non-A1 subclones (BU, 0.59; P < .001). Therefore, we have great confidence in affirming that the BU for the population of BECC A1 is ~3–4 times higher than that observed for the other 3 groups analyzed (figure 2A). Only 2% of the BECC A1 isolates were classified as nonadherent, whereas adherences of 24%, 20%, and 14% were verified for MSSA isolates, sporadic MRSA clones, and BECC non-A1 subclones, respectively. The major contributor to the increase in the BU for the BECC A1 population was that most (74%) isolates were strong biofilm producers (figure 2B).

The percentage of adhesion of BECC A1 isolates in airway cells (6.4%) was 7–10 times higher (P < .001) than that of sporadic MRSA clones (0.87%) or MSSA isolates (0.67%) (figure 3A). Also, there was an extraordinary difference (P < .001) in the ability of BECC A1 isolates to invade these airway cells, compared with that of the other populations (figure 3B). Only 8 of 32 S. aureus isolates analyzed were defined as highly invasive (percentage of invasion, ≥10%), and all of them displayed the A1 PFGE pattern. In addition, the other 2 BECC A1 isolates tested were defined as invasive (percentage of invasion, 6.1% and 4.1%). In contrast, the majority of sporadic MRSA clones and MSSA isolates (20 of 22) were classified as poorly invasive (9) or noninvasive (11). Only 2 sporadic MRSA clones were invasive (percentage of invasion, 5.7% and 2.0%). The high level of invasion by BECC A1 isolates was verified only during the log phase (figure 3C).

Electron microscopy studies, performed with the BECC A1 isolate GV91, showed that the bacterial adherence to airway cells was unique, in the sense that the adhesion occurred in a delicate contact between the bacterial hairlike structures with the apical surface of 2 or 3 microvilli (figure 4B). Indeed, the enhanced invasion involved a massive collapse of microvilli that was characterized by a短ening and loss of these structures (figure 4A and 4C). After 1 h of contact with epithelial cells, a large number of bacteria were internalized, and most of them remained within membrane-bound vesicles, where many dividing cells (with septal rings) were visualized (figure 4D).

Treatment of the bacteria with soluble Fn decreased the percentage of invasion from 31% to 1.2% for isolate GV69 and from 43.7% to 1.8% for isolate GV91 (P < .001) (figure 5A).
The entry of these 2 BECC A₁ isolates was also impaired by treatment of monolayers with RGD peptide \((P < .001)\). In addition, when protease was used, the release of proteinaceous structures from the bacterial surface abolished the bacterial entry \((P < .001)\) (figure 5A).

The effect of the environmental conditions on bacterial growth also reduced the high level of invasion by BECC A₁ isolates \((P < .001)\) (figure 5B). Invasion by BECC A₁ isolates decreased to 0.62% (isolate GV69) and 0.13% (isolate GV91) when the bacteria were grown at pH 5.0. Similarly, the addition of dextrose to the culture medium resulted in a reduction of bacterial invasion to 3.4% and 16.3% (isolates GV69 and GV91, respectively; \(P < .001)\). Ionic changes in the composition of the medium, such as increased magnesium or sodium concentrations, also blocked invasion \((P < .001)\). When these 2 isolates were grown at 30°C or 40°C, a similar strong inhibition of bacterial invasion was verified (figure 5B).

Finally, the percentage of adhesion of isolate GV91 to solid-phase Fn (24.2%) was increased, compared with that of strain RN6390B (15.3%), for the experiments performed at log phase \((\text{OD}_{610}, 0.4; P = .048)\). This increase in adhesion of isolate GV91 was even greater (14% for GV91 and 2% for RN6390B) when the tests were performed at stationary phase \((\text{OD}_{610} \uparrow \text{up to} 1.8; P < .001)\) (figure 6). As expected, the adherence of the \(agr^-\) strain RN6911 to solid-phase Fn was constant in the log (13.5%) and stationary (11.4%) phases.

**DISCUSSION**

We molecularly characterized MRSA isolates from the João Barros Barreto University Hospital. In 1995, the incidence of BECC isolates displaying the dominant A₁ PFGE pattern was low, but the frequency of BECC A₁ isolates increased until these isolates supplanted much of the non-A₁ MRSA population. Once all non-A₁ MRSA isolates collected in this hospital had shown that the predominant isolate of the BECC to produce biofilm and to invade the epithelial cell monolayer was confirmed for BECC A₁ isolates. Although it is difficult to translate our in vitro studies to what exactly occurs during an in vivo pathogenic process, it follows logically that the enhanced biofilm production, adhesion, and invasion of epithelial cells displayed by BECC A₁ isolates have implications for their persistence, prevalence in nosocomial infections, and widespread appearance over large geographic areas.

Studies investigating the early events in invasion of epithelial cells by \(S. aureus\) showed that the entry of the bacterium involved Fn-binding proteins \([30, 32]\) and that the interaction of \(S. aureus\) with Fn led to induction of signal transduction, tyrosine kinase activity, and cytoskeletal rearrangement \([32]\). Moreover, it was shown that both Fn and \(\beta 1\) integrins were also required for maximum uptake \([32]\). In the present study, we verified that the high level of invasion displayed by BECC A₁ isolates was totally dependent on the presence of accessible Fn-binding domains on the bacterial surface. Furthermore, the treatment of bronchial cells with RGD peptide significantly impaired the enhanced invasion by BECC A₁ isolates, which suggests that integrins served as host cell receptors for the successful bacterial entry into these cells. It was also verified that BECC A₁ isolates bound more efficiently to solid-phase Fn than an \(agr^-\) strain (RN6390B). In addition, we observed that, although the Fn-binding activity of BECC A₁ isolates decreased, it was still higher in the stationary
phase than that of an agr\textsuperscript{r} strain; this is unusual among \textit{S. aureus} clinical isolates, because the expression of Fn-binding proteins is down-regulated by agr in this growth phase [24]. All the evidence suggests that the mechanism associated with the enhanced invasion verified for BECC A\textsubscript{1} isolates was similar to that of the poorly invasive process shown by most \textit{S. aureus} isolates in the sense that it involves both Fn-binding activity and RGD-dependent integrins.

It was reported elsewhere that an agr\textsuperscript{r} mutant (RN6911) displayed enhanced adhesion and invasive properties [33]. Indeed, agr attenuation was verified in an epidemic MRSA clone (non-BECC C-MRSA-3) from Canada that also displayed increased Fn-binding activity [34]. It is remarkable that a relationship between agr dysfunction and persistent bacteremia due to MRSA infections was recently demonstrated in a study published elsewhere [35]. We are now conducting experiments to assess the involvement of global regulators, including agr RNAIII and SarA protein, in the enhanced colonization ability of BECC A\textsubscript{1} isolates. The preliminary results of Northern blotting experiments (with a RNAIII-specific probe) of isolate GV91 revealed an agr attenuation for this BECC A\textsubscript{1} subclone. Time course experiments showed RNAIII expression was 2-fold diminished for isolate GV91, compared with that of the agr\textsuperscript{r} strain RN6390B, and both had agr polymorphism type I. This result was paralleled by an increase in Fn-binding activity (A.M.S.F., B.T.F.-C., and L.A.T., unpublished data).

Another interesting finding in the present study was that, when BECC A\textsubscript{1} isolates were grown under different environmental conditions, the effect of these variables on high-level invasion was dramatic. That these variables had an effect indicates that the high level of invasion by these predominant MRSA isolates is tightly coordinated via a bacterial sensory system, as was verified for other important virulence traits [24, 36]. In conclusion, our results clearly show that the predominant variant of the BECC displays differential in vitro properties (adhesion and invasion) that are commonly associated with the bacterial ability to promote infections. This finding supports the hypothesis that the dominance of specific multidrug-resistant \textit{S. aureus} clones, in a particular epidemiological scenario, seems to result from both the antimicrobial selective pressure and the dynamic association of different factors involved in the bacteria-host coevolution that culminate in the selection of well-adapted pathogens [37].

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References