Increased Colonization of Indwelling Medical Devices by Quorum-Sensing Mutants of *Staphylococcus epidermidis* In Vivo

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Infections with the leading nosocomial pathogen *Staphylococcus epidermidis* are characterized by biofilm development on indwelling medical devices. We demonstrate that the quorum-sensing regulator *agr* affects the biofilm development of *S. epidermidis* in an unexpected fashion and is likely involved in promoting biofilm detachment. An isogenic *agr* mutant showed increased biofilm development and colonization in a rabbit model. In addition, nonfunctional *agr* occurred more frequently among strains isolated from infections of joint prostheses. Lack of functionality was based on mutations, including insertion of an IS256 element. Relative to other bacterial pathogens, quorum sensing in *S. epidermidis* thus has a different role during biofilm development and biofilm-associated infection. Our results indicate that disabling *agr* likely enhances the success of *S. epidermidis* during infection of indwelling medical devices. The permanent elimination of quorum-sensing regulation used by *S. epidermidis* represents a surprising and unusual means to adapt to a certain environment and type of infection.

Biofilm formation has been recognized during the past decade as an enormously important factor contributing to the virulence of pathogenic bacteria in chronic infections [1]. Examples are the biofilms of *Pseudomonas aeruginosa* in the lungs of patients with cystic fibrosis [2], dental-plaque biofilms produced by several *Streptococcus* species [3], and the biofilms caused by *Staphylococcus epidermidis* and *S. aureus* on indwelling medical devices [4]. A biofilm represents a surface-attached agglomeration of cells that are usually embedded in a heterogeneous matrix. Biofilm formation constitutes a critical virulence factor, because it causes resistance against antibiotics and attacks by the human immune system [5]. The formation of a biofilm has been described as a multistep process that involves an initial attachment step, a proliferation phase, and a detachment phase [6]. Whereas initial binding to abiotic surfaces in vitro is mostly based on hydrophobic interaction, primary attachment during infection occurs via the binding of dedicated bacterial surface receptors to host matrix proteins [7]. The proliferation phase is characterized by the production of extracellular matrix molecules (e.g., exopolysaccharides) and the formation of a defined structure. A mature biofilm reveals fluid-filled channels that ensure the provision of nutrients and oxygen to all cells in the biofilm [1]. We have little understanding about how the formation of the 3-dimensional structure in a biofilm is controlled. However, bacterial cell-to-cell communication, in particular quorum sensing, is believed to be involved [8]. The factors that cause the detachment of cell clusters in the final phase of biofilm formation, a mechanism of utmost importance for the spread of an infection, are mostly unknown.

Quorum-sensing systems regulate the shift in protein expression when bacteria react to changing environmental conditions that arise from an increased population density [9]. They use a secreted signal called a pheromone to sense the status of cell density. As the
colonization of infection sites proceeds, with a shift from planktonic to sessile growth and from low to high cell density, the targets of quorum-sensing regulation are often composed of factors important for bacterial colonization and survival. Quorum-sensing systems in several bacteria have been shown to regulate virulence factors, and there are also indications for an influence on biofilm formation [8, 10–12]. Whereas several gram-negative bacteria possess ≥2 quorum-sensing systems [13], staphylococci have only 1 well-characterized quorum-sensing system, named agr for “accessory gene regulator” [14]. We do not know yet whether a further quorum-sensing system that uses a pheromone called autoinducer-2 [15] plays a role in quorum-sensing control in staphylococci. The sequence of agr is highly variable, and specificity groups exist that are defined by a common, posttranslationally modified peptide pheromone [16]. The staphylococcal quorum-sensing system shows the unique phenomenon of cross-inhibition: pheromones from the self induce, and those not from the self block, the quorum-sensing response [17, 18]. Therefore, pheromone-based drugs have been proposed to control quorum sensing and, thereby, the production of virulence factors, in staphylococci [17–19]. In general, quorum-sensing systems have been the focus of the search for new antibacterial drug targets, and so-called quorum-sensing blockers have been proposed to control quorum sensing and, thereby, to predominantly belong to different restriction fragment–length polymorphism types and are therefore essentially nonclonal [23]. A clinical, biofilm-forming S. epidermidis isolate [24] and its isogenic agr mutant [11], in which the entire agr locus has been replaced by a spectinomycin resistance cassette, were used for confocal laser scanning microscopy (CLSM), cell adherence, and animal experiments. The reporter plasmid used to visualize activity of agr was constructed by cloning the P3 promoter region of the agr system of S. epidermidis in front of a gene coding for enhanced green fluorescent protein (EGFP; Clontech) on an Escherichia coli/Staphylococcus shuttle plasmid (pRB473) [25]. Strains carrying the “empty” plasmid pRB473 were used as controls. Animal experiments were performed according to US Department of Health and Human Services guidelines, and the protocol was approved by the Animal Care and Use Committee of Rocky Mountain Laboratories.

**Analysis of agr functionality.** Analysis of agr functionality was performed by determining the presence of δ-toxin, as described elsewhere [26], with the following modifications. δ-Toxin was detected in bacterial culture medium by reversed-phase high-performance liquid chromatography (HPLC) by use of a Zorbax SB-C8 2.3 × 30 mm column (Agilent Technologies). Then, 20 μL of culture medium that had been cleared from cells by centrifugation at 21,000 × g for 5 min was directly injected onto the column. After injection, the column was first washed with 10% buffer B for 2.5 min, followed by 50% buffer B for 3.5 min (buffer A, 0.1% trifluoroacetic acid [TFA] in water; buffer B, 0.1% TFA in acetonitrile). A gradient of 50–90% buffer B was then applied over the course of 10 min. The column was run at a flow rate of 0.4 mL/min. The identity of δ-toxin was controlled by its mass, using liquid chromatography–mass spectrometry (LC-MS). The LC-MS system consisted of an Agilent 1100 series HPLC system connected to an Agilent Trap VL ion trap–type mass spectrometer.

### MATERIALS AND METHODS

**Bacterial strains.** The strains isolated from joint prostheses infections and control strains were obtained from N. El Solh (Institut Pasteur, Paris, France). They were collected from hospitals in the Paris area and from the skin of healthy individuals in the same geographic region. The strains have been described to predominantly belong to different restriction fragment–length polymorphism types and are therefore essentially nonclonal [23]. A clinical, biofilm-forming S. epidermidis isolate [24] and its isogenic agr mutant [11], in which the entire agr locus has been replaced by a spectinomycin resistance cassette, were used for confocal laser scanning microscopy (CLSM), cell adherence, and animal experiments. The reporter plasmid used to visualize activity of agr was constructed by cloning the P3 promoter region of the agr system of S. epidermidis in front of a gene coding for enhanced green fluorescent protein (EGFP; Clontech) on an Escherichia coli/Staphylococcus shuttle plasmid (pRB473) [25]. Strains carrying the “empty” plasmid pRB473 were used as controls. Animal experiments were performed according to US Department of Health and Human Services guidelines, and the protocol was approved by the Animal Care and Use Committee of Rocky Mountain Laboratories.

**CLSM.** S. epidermidis wild-type (wt) and agr mutant strains were inoculated from precultures grown overnight at a dilution of 1:200 in 8-well polystyrene chambers (Lab-Tek II; Nunc). After 24 h of incubation at 37°C, supernatants were gently removed, and biofilm layers were washed with saline and resuspended in 400 μL of saline. The expression of agr was monitored in biofilm, wash, and the supernatant fraction. Bacteria in biofilm cultures were visualized by use of the LIVE/DEAD BacLight bacterial viability staining kit or acridine orange stain (1 mg/mL), as described by the manufacturer (Molecular Probes). Images (512 × 512, 8 bit) were acquired on a Zeiss LSM 5 Pascal laser-scanning confocal unit (Carl Zeiss) equipped with an argon laser, a helium-neon laser, and an Axiocam 100 microscope with a 63 × 1.4–NA oil-immersion objective. Zeiss 3D (Image VisArt) software was used for the 3-dimensional visualization of biofilm structures.

**Human cell culture and adherence assay.** A2058 human skin epithelial cells (ATCC CRL-1147) were used to compare the level of adherence of S. epidermidis wt and agr mutant strains. Cells were cultured on plastic coverslips placed in 24-well tissue-culture plates with 1 mL of Dulbecco’s modified Eagle medium (DMEM) and 4 mmol/L l-glutamine supple-
mented with 10% fetal bovine serum (FBS; Invitrogen) at 37°C in an atmosphere with 5% CO₂. Cells were grown to semiconflueney (4 × 10⁵ cells/well) without antibiotics. *S. epidermidis* cells were grown in tryptic soy broth (TSB) medium supplemented with 0.5% glucose to the exponential growth phase (OD₆₀₀ 0.4). Bacteria were harvested, washed once with cEBS salt solution (0.27 g/L CaCl₂, 0.4 g/L KCl, 0.1 g/L MgSO₄·7H₂O, 6.8 g/L NaCl, 2.2 g/L NaHCO₃, 0.12 g/L NaH₂PO₄·H₂O, and 1 g/L glucose), and resuspended in cEBS salt solution. A2058 cells were inoculated with 10⁶ cfu of *S. epidermidis*/well in DMEM plus 10% FBS. Tissue-culture plates were centrifuged for 4 min at 200 g, to promote contact between bacteria and epithelial cells, and incubated for 2 h at 37°C with 5% CO₂. Wells were washed 3 times, to remove nonadherent bacteria. A2058 cells were fixed for 1 h in 1 mL of PBS that contained 2% paraformaldehyde and were stained with crystal violet. The number of cell-associated bacteria was determined by counting 50 random A2058 cells in 4 different fields with a light microscope. Experiments were performed in triplicate, and results were expressed for each experiment as the mean (± SE) number of *S. epidermidis* cells/A2058 cell.

**RNA isolation, cDNA synthesis, and reverse-transcription polymerase chain reaction (RT-PCR).** RNA was isolated as follows. For planktonic cells and loosely attached biofilm cells, a FastPrep BLUE (Q-BioGene) kit and a standard protocol were used with the following modifications. In a 2-mL FastPrep tube, 500 μL of chaotropic RNA-stabilizing agent–BLUE, 500 μL of acid phenol, and 100 μL of chloroform were added. The tubes were placed in a high-speed homogenizer (Bio101/Savant Instruments) and run at speed 6.5, 35 times—this cycle was repeated twice, with an interval of 5 min on ice. Immediately after shaking, the tubes were chilled on ice for 5 min and centrifuged. The top layer was removed, and the remaining liquid was transferred into a new tube and extracted with chloroform until the middle protein layer was no longer visible. The resulting RNA was precipitated with isopropanol and washed. The remaining DNA was removed by use of RNase-free DNase I (Amersham Biosciences). The reaction product was extracted with phenol until the middle layer was no longer present, and RNA was precipitated. For biofilm cells, RNA isolation was performed as described above, with an additional shaking step at the beginning in a high-speed homogenizer at speed 5.5, 35 times—this cycle was repeated twice, with an interval of 5 min on ice.

Synthesis of cDNA and RT-PCR were performed according to a standard protocol described by Chaussee et al. [27]. Oligonucleotide primers and probes were designed by use of Primer Express 2.0 software (PE Biosystems) and synthesized by Applied Biosystems. Standard curves for RT-PCR were determined for each gene, with purified chromosomal template DNA at concentrations of 0.001–10 ng/mL, and 16S rRNA was used as a control.

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Figure 1. Influence of a quorum-sensing (agr) mutation and quorum-sensing blockers on biofilm depth. Biofilms of *Staphylococcus epidermidis* after 24 h were visualized by confocal laser scanning microscopy (CLSM). agr, isogenic agr mutant strain; wt, *S. epidermidis* wild-type strain; wt + inhibitor, wt strain with the addition of 100 nmol/L inhibiting pheromone of *S. aureus* agr subgroup type 4 [42]. ***P<.0001 vs. wt. A, Biofilm depths, results from 6 independent experiments, using 5 values/experiment. B, Representative CLSM images. Yellow bar, 10 μm.

**Animal model of indwelling medical device-related infection.** Female New Zealand white rabbits were tranquilized for restraint with acepromazine (0.5 mg/kg), administered intravenously via the marginal ear vein, or ketamine (20 mg/kg) combined with acepromazine (1 mg/kg), administered intramuscularly. The skin was surgically prepped via clipping the hair, followed by surgical scrub with betadine and an alcohol rinse (repeated 3 times), and then coated with betadine solution. This was followed by subcutaneous (sc) implantation, via a small stab incision and sterile forceps, of 2-cm pieces of medical tubing (Tygon silicone tubing; Saint Gobain) on the dorsum, near the midpoint of the body. The tubing pieces were incubated in solutions that contained *S. epidermidis* stationary-phase cells (5 × 10⁶/mL) for 10 min and were briefly rinsed in sterile saline before insertion into the animals. One week after inoculation, the animals were killed by exsanguination under deep surgical anesthesia (ketamine/xylazine). The study protocol was approved by the Animal Care and Use Committee of Rocky Mountain Laboratories. The isolation of adherent staphylococcal cells from medical tubing was performed as fol-
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RESULTS

Quorum-sensing control–limiting biofilm expansion in S. epidermidis. CLSM represents the state-of-the-art method to investigate the in vitro formation of biofilms. We constructed an EGFP reporter fusion to measure the expression of the agr P3 promoter. This promoter drives the expression of RNAIII, the regulatory molecule of the agr system, which determines agr activity [28, 29]. S. epidermidis isolates that contained the resulting promoter test plasmid and an isogenic agr deletion mutant [11] were used to investigate the role that quorum sensing plays in biofilm formation. For our experiments, we used a static biofilm model, as described by Yoon et al. for P. aeruginosa biofilms [30]. Such a model appeared to be more appropriate than a dynamic model, because biofilms formed by S. epidermidis on indwelling medical devices are usually not exposed to dynamic flow [31].

We found that the agr mutant formed a significantly thicker biofilm than the wt strain (P < .0001, 2-tailed t test) (figure 1). In addition, a cross-inhibiting agr pheromone, as an example

Figure 2. Spatial pattern of quorum-sensing (agr) expression in an Staphylococcus epidermidis biofilm. A 3-dimensional view of an S. epidermidis biofilm after 24 h, stained with a bacterial LIVE/DEAD kit, shows live cells in green and dead cells in red. The expression of agr was monitored by the fluorescence of enhanced green fluorescent protein (EGFP), whose gene was cloned under control of the S. epidermidis agr P3 promoter. EGFP fluorescence is shown in yellow (artificial color). agr, isogenic agr mutant; wt, S. epidermidis wild-type strain.

Figure 3. Expression of the quorum-sensing agr system in planktonic cells and loosely attached biofilm cells. The expression of agr was monitored by expression of enhanced green fluorescent protein (EGFP) under control of the Staphylococcus epidermidis agr P3 promoter. Green color shows EGFP fluorescence. A, planktonic cells; C, loosely attached biofilm cells that were released from the biofilm surface by gentle washing; B and D, corresponding controls using the isogenic agr mutant strain with the same vector.
of a quorum-sensing blocker, significantly enhanced the thickness of biofilms ($P < .0001$, 2-tailed $t$ test). These results are in accordance with those of our earlier studies, which were based on simple, semiquantitative biofilm assays and suggested that biofilm formation was controlled by $agr$ [11]. Of note, $agr$ expression was restricted to the upper and most-exposed regions of the biofilm—that is, to the biofilm/liquid interface (figure 2). In contrast, there was no detectable expression of $agr$ in deeper layers of the biofilm. It is of importance that most bacterial cells were viable in all layers of the biofilm, which indicates that the observed difference of the expression of $agr$ in the biofilm was not due to a difference in bacterial cell death. Similar to exposed biofilm layers, planktonic cells and cell clusters loosely attached to the surface of the biofilm revealed high expression of $agr$ (figure 3).

The emission of fluorescence by green fluorescent protein is oxygen dependent. Although this oxygen dependence has been shown to not alter fluorescence intensity in biofilms with a depth of <50 $\mu$m [32], a biofilm thickness greater than that in our experiments, we also tested $agr$ expression by use of RT-PCR of RNAIII. RNAIII expression was increased by 6-fold in the loosely attached biofilm cell fraction, compared with that in the biofilm body, confirming the results obtained by analysis of promoter activity.

In conclusion, the enhanced biofilm formation of an isogenic $agr$ mutant, restriction of $agr$ expression to planktonic, loosely attached, and exposed cells of the biofilm, and the contrasting lack of $agr$ expression in the biofilm body suggest that $agr$ might be involved in the biofilm detachment processes.

**Increased adhesion to epithelial cells in an S. epidermidis quorum-sensing mutant.** *S. epidermidis* is a commensal of human skin, and most *S. epidermidis* infections are caused by skin-colonizing strains that penetrate the protective layers of the skin or mucosal membranes [33]. Epithelial cells thus represent an important cell type that *S. epidermidis* must be able to colonize to establish an infection. Therefore, we investigated the capacity of *S. epidermidis* wt and an isogenic $agr$ mutant to adhere to human epithelial cells. According to results obtained by use of *S. aureus*, lack of $agr$ expression during early growth phases results in the increased expression of host matrix protein–binding proteins [34]. Similarly, $agr$ controls the expression of surface proteins in *S. epidermidis* [35]. Accordingly, the $agr$ mutant revealed significantly increased binding ($P < .0001$, 2-tailed $t$ test) (figure 4) to epithelial cells, which suggests that decreased quorum-sensing activity enhances the colonization step of *S. epidermidis* infection.

**S. epidermidis quorum-sensing mutant showing higher infectivity in a rabbit model of device-related infection.** We used a rabbit model of device–related infection to investigate the pathogenicity of an *agr* mutant in biofilm-associated infection. Bacteria adherent to the implants and in the surrounding tissue were counted 1 week after infection. The *agr* mutant had a higher capacity to cause infection on the implants ($P = .0012$, 2-tailed $t$ test) (figure 5A). In contrast, the wt was found more frequently in tissue samples, whereas the *agr* mutant was less invasive ($P = .0469$, 2-tailed $t$ test).
Table 1. Functionality of the agr system among strains obtained from infections of joint prostheses.

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<thead>
<tr>
<th>Strain (no.)</th>
<th>agr status(a) (%)</th>
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<tr>
<td>Clinical (53)</td>
<td>Functional 34 (64) Nonfunctional 19 (34)</td>
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<tr>
<td>Control(b) (21)</td>
<td>20 (95) 1 (4.7)</td>
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\(a\) The functionality of agr was assessed by high-performance liquid chromatography of the \(\delta\)-toxin.

\(b\) Control strains were from the skin of healthy individuals from the same geographic region.

(figure 5B). Thus, the deletion of agr significantly increased success in the biofilm-associated colonization of indwelling devices, which reflects our in vitro results on the impact of agr on biofilm formation. On the other hand, increased invasiveness of the wt strain is in accordance with our previous results regarding the up-regulation of extracellular virulence factors by agr in S. epidermidis, which are deemed to be important for tissue invasion [35].

**Correlation of the lack of quorum-sensing activity in clinical S. epidermidis strains with origin from prosthesis infection and based on mutations in the agr system.** In vitro, agr mutants arise with a certain frequency in S. aureus [36]. We therefore hypothesized that agr mutants also occur naturally in S. epidermidis and wanted to test whether such mutants can be found in device-related infections with S. epidermidis and, on the basis of our animal studies and in vitro results, possibly correlate with increased infectivity. To assess agr functionality, we determined the expression of \(\delta\)-toxin, by HPLC, as described elsewhere [26]. The \(\delta\)-toxin is encoded within RNAIII and is therefore a direct sign for agr activity [29]. A total of 53 S. epidermidis strains isolated from patients with infections of joint prostheses and 21 S. epidermidis strains isolated from the skin of healthy individuals were assayed. These strains have been shown elsewhere to have low clonal relatedness [23]. Nonfunctionality of the quorum-sensing system was significantly more frequent among the clinical isolates (36%) than among strains from healthy individuals (4.7%) \((P = .0066, \chi^2\) test) (table 1). These results suggest that the permanent disabling of agr enhances the success of S. epidermidis in biofilm-associated infection. Although we have reported previously that strains with a nonfunctional agr system can be found in S. aureus [12], to our knowledge, the present report is the first to show that this mechanism also occurs frequently in S. epidermidis and, even more important, that strains with a nonfunctional agr system have increased virulence in the biofilm-associated infection of indwelling devices.

To determine whether agr nonfunctionality was caused by DNA mutations within the agr system rather than by regulatory processes, we sequenced the agr system of the functional and nonfunctional strains. Among the nonfunctional strains, 6 revealed frameshift mutations, of which 4 occurred in agrC and 2 in agrA. Three strains had an IS256 insertion in agrC, and 3 showed multiple point mutations in agrC leading to several nonconservative amino-acid exchanges. Thus, for many strains, we detected DNA mutations as a very likely cause for the lack of agr expression. Also, because the agr-negative phenotype of the strains was stable, we conclude that phase variation was not the reason for the observed changes. It is of interest that most mutations occurred in the agrC gene, which might therefore represent a preferred site for mutations that lead to a disabled agr system. Of importance, no changes within the genetic information for \(\delta\)-toxin were found, which confirms that the measurement of \(\delta\)-toxin is a valid means to assess the functionality of agr.

**DISCUSSION**

It is commonly believed that a defined structure is critical for the viability of a biofilm and, as a consequence, for the virulence of biofilm-forming pathogenic bacteria during biofilm-associated infection [1]. Quorum sensing as a means of cell-to-cell communication has been proposed to trigger the formation of cell agglomerations and water-filled channels in a biofilm via cell density-dependent controls [8]. We have described that the agr quorum-sensing system of staphylococci plays an unexpected role in biofilm formation by S. epidermidis. On the basis of our results, we propose a model of agr in biofilm formation that involves agr activation at the biofilm/fluid interface and agr-dependent regulation of factors that promote cell detachment. The restriction of agr expression to exposed biofilm layers contrasted what would have been predicted from an exclusively quorum-sensing control, which should have caused high activity of agr in dense regions of the biofilm. However, it is known from S. aureus that other regulatory systems can induce the expression of agr [37]. Several factors in addition to cell density (e.g., oxygen concentration and other environmental factors) might thus influence the expression of the agr system indirectly in a complicated network of regulators, which we are far from understanding.

We know more about the factors that might mediate the impact of agr on biofilm formation. In a previous study, we showed that the secreted, detergent-like peptide \(\delta\)-toxin, whose expression is entirely dependent on agr expression, can inhibit biofilm formation in vitro [11]. Artificial surfactants have been demonstrated to interfere with the attachment phase of S. epidermidis biofilm formation [38]. However, our previous results suggested that \(\delta\)-toxin, in contrast, exerts its inhibitory effect during later stages of biofilm formation [11]. The spatial pattern of agr expression in S. epidermidis biofilms that we have described here contributes further to our understanding of the role that \(\delta\)-toxin plays in biofilm formation. At the biofilm/
fluid interface, where *agr* is expressed, a film of amphipathic δ-toxin molecules may form, inhibiting hydrophobic interaction between bacterial cell surfaces and lowering surface tension. As a consequence, bacterial cells can separate, clusters may detach, and channels in the biofilm are able to form. Similarly, in *Bacillus subtilis*, the amphipathic peptide surfactin influences the biofilm structure during fruiting body formation and is controlled by a regulator of sporation [39]. Thus, detergent-like peptides that are controlled by cell-to-cell communication might emerge as a new class of determinants of biofilm structuring and dissemination.

During the biofilm-associated infection of indwelling medical devices, virulence is based on the successful adhesion and proliferation of the infecting pathogen. Our in vitro results prompted us to investigate the role that *agr* plays in biofilm-associated infection. We found that the isogenic *agr* deletion mutant colonized implants more frequently in a rabbit model of indwelling device–related infection, compared with the wt. Furthermore, the nonfunctionality of *agr* also occurred naturally and was based on mutations in *agr*. The increased frequency of *agr* mutants found among strains isolated from infections of indwelling medical devices suggests that the conditions needed to colonize the implant select for quorum-sensing mutants. However, the production of several degradative exoenzymes is reduced in an *agr* mutant of *S. epidermidis* [35], which might be the cause for decreased invasiveness in the animal infection model. When disabling *agr*, the bacteria thus appear to “compromise” to lower the expression of aggressive virulence factors for the sake of producing colonization factors and to enhance biofilm formation. The permanent abandonment of quorum-sensing control as a crucial central regulatory system represents a hitherto unknown means to control colonization and an unexpected way to adapt to a specific environment and type of infection.

In general, *agr* control of biofilm formation in *S. epidermidis* is reminiscent of the roles of quorum-sensing systems described in gram-negative bacteria, as in all systems that influence biofilm structure. However, there seem to be species-specific differences—quorum-sensing mutants showed a thinner biofilm in *P. aeruginosa* [8] but a thicker biofilm in *Vibrio cholerae* [10, 40] and *S. epidermidis*. The similar impact on biofilm depth of the *S. epidermidis* and *V. cholerae* quorum-sensing mutants in vitro would suggest a consistent behavior during biofilm-associated infection. Still, in contrast to impaired colonization found for the *V. cholerae* hapR mutant, the *S. epidermidis* *agr* mutant colonized implants more successfully than did the wt, which suggests that the role of quorum sensing in biofilm formation differs in all these pathogens. It is of interest that, although the lack of the production of detergent-like peptides might result in a less structured biofilm in *S. epidermidis* than in other bacteria, this effect does not cause decreased colonization of indwelling medical devices. It might, however, be important for the life of *S. epidermidis* on the skin as its natural habitat.

Quorum-sensing blockers have been demonstrated to successfully inhibit in vitro biofilm formation by *P. aeruginosa* [41]. In contrast, our present data, which show the increased virulence of an *agr* mutant of *S. epidermidis* in a rabbit model and during human infection, emphasize our previous suggestion [11] to not use such drugs for the treatment of biofilm-associated *S. epidermidis* infection.

In conclusion, we have shown that *agr* influences the 3-dimensional structure of a biofilm, most likely by affecting detachment processes. However, during biofilm-associated infection of indwelling medical devices, it seems to be of advantage for the bacteria to disable the quorum-sensing system, which leads to the increased production of colonization factors and biofilm formation and, ultimately, to a greater success in establishing infection on implants. Our results show that interfering with the function of a quorum-sensing system might lead to increased, rather than decreased, virulence by enhancing bacterial colonization.

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**References**


