Relation between Antibiotic Susceptibility and Ultrastructure of *Staphylococcus aureus* Biofilms on Surgical Suture

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Abstract

Background: Infectious biofilms are recalcitrant to antimicrobial therapy, but the mechanism(s) responsible for the greater resistance are unclear. Experiments were designed to clarify the association between antibiotic resistance and biofilm ultrastructure.

Methods: *Staphylococcus aureus* was cultivated for 24 h on silk suture, where robust biofilms formed. Initial experiments compared the susceptibilities of planktonic (free-living) cells and mechanically dispersed biofilm cells to ampicillin, oxacillin, and vancomycin. Antibiotics in bactericidal concentrations were then incubated overnight with 24-h biofilms, and subsequent assays determined the viability of cells in mechanically dispersed biofilms, biofilm metabolic capacity and biomass, and biofilm ultrastructure (scanning electron microscopy).

Results: Planktonic and biofilm cells had similar intrinsic antibiotic susceptibility. Nonetheless, a stable population of bacteria remained viable after biofilms were incubated with inhibitory drug concentrations, although biofilm metabolic capacity often was not detected, and biomass generally was reduced. Electron microscopy revealed that control (no drug) biofilms consisted primarily of bacterial clusters amid fibrillar elements. Antibiotic-treated biofilms had some staphylococci with smooth cells walls similar to planktonic cells, but other cocci were encased in extracellular material. This material was more abundant in antibiotic-treated than in control biofilms.

Conclusions: In the presence of high antibiotic concentrations, dense extracellular material may inhibit interaction of antibiotics with their bacterial targets.

BIOFILMS ARE POPULATIONS of micro-organisms growing on a surface that are surrounded by a complex extracellular polymeric substance (EPS) composed of proteins, glycoproteins, glycolipids, polysaccharides, and extracellular DNA [1]. There is growing awareness that many infectious diseases are based on bacterial growth as a biofilm [2,3]. These infections are diverse and include colitis, vaginitis, urethritis, conjunctivitis, otitis, dental infections, biliary tract infections, prostatitis, osteomyelitis, burn wound infections, endocarditis, lung infections in cystic fibrosis and ventilated intensive care unit patients, and infections of indwelling devices such as catheters, joint prostheses, heart valves, and stents [3,4]. Bacteria within a biofilm often are more antibiotic resistant than are planktonic (free-living) bacteria [4–7], and biofilm infections are recalcitrant to antibiotic therapy.

Unfortunately, there are no universally accepted methods for studying the antibiotic susceptibility of bacteria in biofilms. Lewis [6] pointed out that although antibiotic resistance

typically is defined as the ability of planktonic bacteria to grow in the presence of antibiotic concentrations above the minimum inhibitory concentration (MIC), most biofilm susceptibility studies assess antibiotic-mediated killing of biofilm-associated bacteria, rather than bacterial growth. This is important because clinical microbiology laboratories report the antibiotic susceptibility of actively growing planktonic cells. Historically, it seemed reasonable to suspect that biofilm-associated bacteria have greater antibiotic resistance than their planktonic counterparts, but evidence indicates this is not the case [8]. It is now believed that a small percentage of biofilm cells are persisters that are stochastically generated, highly tolerant of antibiotics, and responsible for the recalcitrance of chronic biofilm infections [9].

We used an in vitro model of *Staphylococcus aureus* biofilms cultivated on surgical suture to clarify factors associated with the antibiotic resistance of biofilms. Nosocomial infections are among the ten leading causes of death in the U.S., and more

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than 20% of nosocomial infections are surgical site infections (SSIs) [10], defined broadly as infections that occur in the operative field after a surgical intervention [11]. There are no definitive data on the incidence of SSIs directly associated with surgical suture, but it is reasonable to assume that a substantial proportion of SSIs involve suture materials, such as the recent report of a chronic (16-mo) SSI caused by a suture-associated polymicrobial biofilm [12]. *Staphylococcus aureus* is one of the most frequent etiologic agents of SSIs (as well as blood stream infections), and the morbidity and mortality rates associated with these infections are of utmost importance for post-surgical and critically ill patients [11,13].

Experiments were designed to clarify the effects of ampicillin (AMP), oxacillin (OXA), and vancomycin (VAN) on *S. aureus* cultivated as planktonic or suture-associated biofilm cells. Although planktonic and mechanically dispersed biofilm-associated cells had similar intrinsic antimicrobial resistance, a stable population of cells within intact biofilms remained viable after incubation with high antibiotic concentrations. Antibiotic-treated biofilms had copious extracellular material that often surrounded individual bacteria, potentially inhibiting antibiotic binding to pertinent bacterial targets.

Materials and Methods

Antibiotic susceptibilities of planktonic and biofilm-associated S. aureus

Staphylococcus aureus ATCC 25923 and RN6390 are wild-type strains that produce biofilms [14,15]. The antibacterial agents consisted of three cell wall-active agents, namely AMP, OXA, and VAN (Sigma-Aldrich, Inc., St. Louis, MO). Using an inoculum of 5×10^5 /mL for both planktonic and mechanically dispersed (described below) biofilm cells, macrodilution susceptibility testing for the MIC and the minimum bactericidal concentration (MBC) followed Clinical Laboratory Standards Institute (CLSI) guidelines [16]. Results are reported from at least two replicate experiments.

To prepare planktonic cells, static cultures were incubated overnight at 37°C in tryptic soy broth (TSB), washed, and re-suspended to the appropriate concentration, determined by densitometry with results confirmed by quantitative plate culture. Suture-associated biofilms were cultivated as described [17]. Briefly, each well of a six-well microtiter plate contained four 1-cm segments of black braided 3-0 silk suture (Ethicon, Inc., Somerville, NJ) suspended in 3 mL of biofilm growth medium, namely 66% TSB supplemented with 0.2% glucose [15]. Each well was inoculated with $\sim 10^5$ *S. aureus* cells and incubated for 24 h at 37°C with gentle rotation. Suture was then gently rinsed, transferred to 2 mL of sterile phosphate-buffered saline (PBS), and sonicated at ~ 50 J at 100% amplitude for 5 sec at 20 kHz using a sonicator (Sonics and Materials, Newtown, CT). The resulting cell suspension was adjusted by densitometry, with the results confirmed by quantitative culture. Sonication had no noticeable effect on bacterial viability, and microscopy confirmed that planktonic and biofilm inocula were similarly dispersed single-cell suspensions.

To assess the antibiotic susceptibility of cells within intact biofilms, *S. aureus* was incubated for 24 h with suture segments as described above. Biofilm-laden sutures were transferred to wells containing fresh medium supplemented with

various concentrations of AMP, OXA, or VAN as high as 100 times the MIC/MBC and incubated overnight at 37°C under static conditions. Control wells contained no antibiotic. Biofilms were assayed for the effect of the antimicrobial agent on the numbers of viable bacteria in biofilm sonicates, as well as on biofilm biomass and metabolic capacity and biofilm ultrastructure.

Biofilm biomass, metabolic capacity, and ultrastructure

Biofilm biomass was measured with crystal violet and with Syto 9 as described [18] with minor modifications. Crystal violet is a basic dye that binds negatively-charged surface molecules, including live and dead bacteria and matrix polysaccharides; green fluorescent Syto 9 is a nucleic acid stain that diffuses passively through bacterial membranes and binds DNA in live and dead cells as well as extracellular DNA in the extracellular matrix [18]. Biofilm-laden sutures were rinsed in PBS, fixed in 99% methanol for 15 min, air-dried, incubated 20 min with 0.5% crystal violet (Fisher Chemical, Pittsburgh, PA), washed, and then incubated for 20 to 30 min in 33% acetic acid to release the crystal violet, with absorbance read at 590 nm. A 5 mM stock solution of Syto 9 (Invitrogen, Carlsbad, CA) was diluted 1:50,000 in PBS and added to rinsed biofilms, which were incubated 45 min in the dark, the fluorescence then being read at 528 ± 10 nm.

Because reduced metabolic capacity appears to contribute to the antibiotic resistance of biofilms, the BioTimer assay [19] was used to assess the effect of antibiotics on the metabolic capacity of *S. aureus* in undisturbed biofilms. Briefly, Mueller-Hinton broth supplemented with 1% glucose and 0.0025% phenol red was added to washed biofilms and incubated at 37°C without shaking. The time of the color change from red to yellow was determined by observation every 30 min for 8 h. A standard curve was generated using known numbers of planktonic bacteria, and results from biofilm cultures were reported as planktonic-equivalent colony-forming units (CFU). Some samples switched from red to yellow after the 8-h observation period, whereas others remained red for 24 h; for mathematical purposes, these samples were assumed to have changed color at 15 h, corresponding to a time when 10 CFU of planktonic *S. aureus* (lower limit of assay detection) has been reported to cause the color switch [19].

To observe biofilm ultrastructure, each of the two *S. aureus* strains was incubated with silk suture for 24 h as described, transferred to fresh medium containing various concentrations of AMP (0.125, 3.125, 12.5 micrograms/mL), OXA (2.5, 6.25, 25 micrograms/mL), or VAN (2, 50, 100 micrograms/mL), with control biofilms containing no antibiotic. After overnight incubation, sutures were processed for scanning electron microscopy (SEM) as described [17,20]. Samples were viewed with a Hitachi S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Inc., Pleasanton, CA) operated at 2.5 kV. Each biofilm was examined for at least 45 min. Results are based on >500 images from 21 samples, each processed in duplicate.

Statistical analysis

Comparisons of two groups were made by the unpaired Student *t*-test and more than two groups by one-way analysis of variance with the Fisher post hoc test. Significance was defined as $p < 0.05$.

TABLE 1. MINIMUM INHIBITORY CONCENTRATIONS AND MINIMUM BACTERICIDAL CONCENTRATIONS OF AMPICILLIN, OXACILLIN, AND VANCOMYCIN FOR STANDARDIZED INOCULA OF *STAPHYLOCOCCUS AUREUS* ATCC 29423 AND RN6390 CULTIVATED AS PLANKTONIC CELLS FROM OVERNIGHT BROTH CULTURE OR AS BIOFILM CELLS MECHANICALLY DISPERSED FROM SILK SUTURE

Concentration (micrograms/mL)		<i>S. aureus</i> ATCC25923			<i>S. aureus</i> RN6390		
		Ampicillin	Oxacillin	Vancomycin	Ampicillin	Oxacillin	Vancomycin
Planktonic	MIC	0.25	0.25	2.00	0.25	0.25	2.00
Planktonic	MBC	0.25	0.50	2.00	0.25	0.25	2.00
Biofilm	MIC	0.25	0.25	2.00	0.25	0.25	2.00
Biofilm	MBC	0.25	0.50	2.00	0.50	0.50	2.00

MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration.

Results

Susceptibilities of planktonic *S. aureus* and biofilm cells to AMP, OXA, and VAN

The MIC/MBCs of AMP, OXA, and VAN were similar for both *S. aureus* strains, and similar MICs and MBCs were ob-

tained using planktonic cells and those dispersed mechanically from intact biofilms (Table 1). According to CLSI breakpoints [16], both *S. aureus* strains were susceptible to these agents, and each agent was bactericidal.

Figure 1A shows the bactericidal effect of AMP incubated overnight with intact *S. aureus* biofilms (rather than dispersed

FIG. 1. Effect of overnight incubation with various concentrations of ampicillin on biofilms formed by 24-h incubation of silk suture with *Staphylococcus aureus* RN6390 or ATCC 25923. (A) Viable colony-forming units (CFUs) from sonicated biofilms ($n=6$). (B) Metabolic capacity of undisturbed biofilms measured as planktonic equivalent CFUs ($n=12$); (C, D) Biofilm biomass measured as absorbance of crystal violet (C; $n=12$) and fluorescence of Syto 9 (D; $n=12$). Significant differences (nearly all at $p<0.01$): *Increased vs. all other concentrations; †Increased vs. 1.25, 3.125, 6.25, and 12.5 micrograms/mL; ‡increased vs. 1.25 micrograms/mL; §increased vs. 3.125, 6.25, and 12.5 micrograms/mL; #increased vs. 12.5 micrograms/mL; \$increased vs. 1.25, 3.125, and 12.5 micrograms/mL.

biofilm cells, as in Table 1). Control (no drug) biofilms contained $\sim 10^{7.3}$ viable *S. aureus*, and overnight incubation with AMP at the MIC or MBC resulted in different degrees of killing for each of the *S. aureus* strains, although substantial numbers of bacteria survived an AMP concentration 100 times the MIC or MBC (Fig. 1A). The metabolic capacity of biofilm cells was affected more profoundly by AMP (Fig. 1B) than was viability per se (Fig. 1A), but relatively high concentrations of AMP were required to inhibit bacterial metabolism (Fig. 1B). Ampicillin decreased the biomass of *S. aureus* biofilms, as measured by both the crystal violet (Fig. 1C) and Syto 9 (Fig. 1D) assays.

Figure 2 shows the effect of OXA on the viability, metabolic capacity, and biomass of undisturbed *S. aureus* biofilms. Again, substantial numbers of cells (10^3 to 10^4) survived treatment with OXA concentrations as high as 100 times the MIC or MBC (Fig. 2A), although biofilm metabolic capacity was undetectable at the higher OXA concentrations (Fig. 2B). Oxacillin decreased the biomass of *S. aureus* biofilms, as assessed by the crystal violet assay (Fig. 2C), but differences found with the Syto 9 assay were not statistically significant (Fig. 2D).

Vancomycin had a similar effect on the cell viability and metabolic capacity of *S. aureus* biofilms, and $\sim 10^{3.6}$ to $10^{4.4}$ bacteria survived. Drug concentrations as great as 100 times the MIC or MBC were tested (Fig. 3A), and biofilm metabolic capacity was undetectable at the higher VAN concentrations (Fig. 3B). Interestingly, using the crystal violet assay, a VAN concentration similar to the MIC/MBC resulted in increased, rather than decreased, biofilm biomass with each of the two *S. aureus* strains (Fig. 3C); and the Syto 9 assay indicated greater biomass with one of the two strains (Fig. 3D).

Ultrastructure of *S. aureus* biofilms incubated with AMP, OXA, or VAN

Established 24-h biofilms were incubated overnight with various concentrations (as much as 100 times the MIC or MBC) of AMP, OXA, and VAN; and samples were processed for SEM. Control (no drug) biofilms thus were biofilms that had been incubated for two days, and representative images are provided in Figure 4. There was much biofilm-to-biofilm variability, and even considerable variability within a single biofilm, but there were clear differences between control and

FIG. 2. Effect of overnight incubation with various concentrations of oxacillin on biofilms formed by 24-h incubation of silk suture with *Staphylococcus aureus* RN6390 or ATCC 25923. (A) Viable colony-forming units (CFU) from sonicated biofilms ($n=6$). (B) Metabolic capacity of undisturbed biofilms measured as planktonic equivalent CFU ($n=15$ or 16). (C, D) Biofilm biomass measured as absorbance of crystal violet (C; $n=14$ – 16) and fluorescence of Syto 9 (D; $n=16$). Significant differences (nearly all at $p<0.01$): *Differs from all other concentrations; †increased vs. 2.5 micrograms/mL; ‡differs from 0, 6.25, 12.5, and 25 micrograms/mL; §increased compared with 12.5 and 25 micrograms/mL.

FIG. 3. Effect of overnight incubation with various concentrations of vancomycin on biofilms formed by 24-h incubation of silk suture with *S. aureus* RN6390 or ATCC 25923. (A) Viable colony-forming units (CFU) from sonicated biofilms ($n=6$). (B) Metabolic capacity of undisturbed biofilms measured as planktonic equivalent CFU ($n=6$). (C, D) Biofilm biomass measured as absorbance of crystal violet (C; $n=9$ or 10) and fluorescence of Syto 9 (D; $n=8-12$). Significant differences (nearly all at $p < 0.01$): *Increased vs. 20, 50, 100, 200 micrograms/mL; †increased vs. 50, 100, 200 micrograms/mL; ‡increased vs. 100 and 200 micrograms/mL; §increased vs. all other concentrations.

antibiotic-treated biofilms. Control biofilms had staphylococcal clusters of various sizes, and cocci appeared to localize preferentially within crevices between individual threads of the suture (Fig. 4A). Extracellular matrix generally appeared as amorphous fibrillar material, but some material seemed more dense and consolidated (Fig. 4).

In general, overnight incubation with AMP, OXA, or VAN had similar effects on the ultrastructure of *S. aureus* suture-associated biofilms. Normal-appearing cocci could be found after treatment with every drug concentration, including concentrations 100 times the MIC or MBC. However, in contrast to control biofilms (Fig. 4), biofilms treated with AMP, OXA, or VAN had numerous discrete clumps of ruffled matrix along, and between, individual suture threads (Figs. 5–7). These clumps contained hollow cup-shaped spaces (Fig. 5C) consistent with the speculation that cocci had once been part of these structures, and occasionally, coccal forms were seen within these ruffled clumps (Fig. 7A). Some staphylococci had normal-appearing cell walls (Fig. 6B, arrows), whereas others had walls encased in fibrillar strands (Figs. 5B; 6B, C; 7A, B) or

in more consolidated-appearing extracellular matrix material (Fig. 7C, D).

Discussion

Although bacteria in biofilms have greater antibiotic resistance, there is substantial evidence that planktonic and dispersed biofilm cells have similar intrinsic antimicrobial susceptibilities [5,6,21,22]. Thus, it was not surprising that *S. aureus* planktonic and dispersed biofilm cells had similar MIC/MBCs for AMP, OXA, and VAN (Table 1). Because substantial numbers of *S. aureus* remained viable after incubation of established biofilms with drug concentrations as much as 100 times the MBC, it might appear that bacteria within intact biofilms were more resistant than bacteria from dispersed biofilms. However, in this study, intact biofilms had comparatively high numbers of viable cells ($>10^7$); and, assuming that effective MBC killing is a $\geq 3 \log_{10}$ reduction, this extent of bacterial killing was achieved with both mechanically dispersed (Table 1) and intact (Figs. 1A, 2A, and 3A)

FIG. 4. Scanning electron micrographs of control (no drug) *Staphylococcus aureus* biofilms cultivated two days on silk suture. (A) Low-magnification view of *S. aureus* RN6390 biofilm showing cocci primarily between individual silk threads (asterisks), with occasional areas of dense matrix material (arrows). (B) More clearly showing cocci embedded in matrix material. (C) Low-magnification view of *S. aureus* 25923 biofilm >10 micrometers in depth. (D) More clearly showing cocci enmeshed in fibrillar strands. Scale bars: A, C = 10 micrometers; B, D = 5 micrometers.

biofilm cells. Although CLSI guidelines define effective bacterial killing as elimination of 99.9% ($\geq 3 \log_{10}$) of viable bacteria [16], this definition may not be relevant for infectious biofilms [4] because the number of viable bacteria in an intact biofilm typically is substantially higher than the inoculum used to perform a susceptibility assay according to CLSI guidelines.

Increasingly high concentrations of AMP, OXA, and VAN did not result in progressive increases in bacterial killing but rather yielded a stable number of resistant biofilm-associated *S. aureus*. Several investigators have treated bacteria with antibiotics and used the survivors in experiments designed to study the properties of putative persister cells [8,9,23,24]. According to this scenario, all antibiotic-treated biofilms had persister cells because 10^2 to 10^5 viable bacteria were recovered after treatment with high concentrations of AMP, OXA, or VAN. Interestingly, Fux et al. [14] reported that the antibiotic resistance of a biofilm correlated with its size, and mechanical disruption of *S. aureus* 25923 biofilm clumps lowered the MBC of OXA more than 1000-fold ($\geq 3 \log_{10}$), yet clumps with as few as ~ 20 bacterial cells had MBCs substantially higher than planktonic cells. Thus, even small biofilm clumps with low bacterial numbers likely contain resistant cells. The question therefore remains: What makes biofilm-associated bacteria more antibiotic resistant than planktonic cells?

Despite the heterogeneity of individual biofilms [1,7], the effects of AMP, OXA, and VAN on biomass were remarkably consistent for the two *S. aureus* strains and in the crystal violet

and Syto 9 assays, and biomass generally was decreased by approximately one-half. A notable exception was the larger biomass found after treatment with a concentration of VAN similar to the MIC or MBC, but not with higher VAN concentrations (see Fig. 3). There is evidence that subinhibitory concentrations of some antibiotics promote biofilm formation, perhaps by facilitating EPS production. For example, Hoffman et al. [25] noted that tobramycin induced biofilm formation by *Pseudomonas aeruginosa* and *Escherichia coli* and suggested that biofilms may form as a defensive reaction to antimicrobial agents. This hypothesis deserves further study.

Micrographs of sutures incubated with *S. aureus* revealed robust biofilms largely localized between strands of the braided silk. In this in vitro model, biofilm structure resulted from bacterial growth on a surface without any contribution of host factors. In control samples without antibiotics, biofilm structure generally consisted of clusters of cocci within fibrillar elements. Clusters of normal-appearing staphylococci also were present (although less frequently) on antibiotic-treated sutures, including those treated with high antibiotic concentrations. Additional structures in antibiotic-treated biofilms included clumps of ruffled matrix containing hollow depressions suggesting that the circular spaces once held staphylococci, and occasionally, cocci were seen within this matrix. Other cocci were covered with matrix that ranged in appearance from loose fibrillar strands to a mixture of fibrils with more consolidated material to a consolidated material that obscured the bacterial cell (Fig. 7C, D). This latter covering often appeared quite dense, making it reasonable to

FIG. 5. Scanning electron micrographs of *Staphylococcus aureus* 25923 cultivated 24 h on silk suture followed by overnight incubation with ampicillin 0.125 micrograms/mL (A, B) or 12.5 micrograms/mL (C). (A) Ruffled matrix material and coccal elements in clumps along individual silk threads, with higher magnification of the inset (B) showing these structures more clearly. Asterisk highlights a depression in the ruffled matrix similar to the size of a staphylococcal cell. (C) Higher-magnification view of ruffled matrix, with asterisks highlighting circular depressions similar to size of staphylococci allowing reasonable contraction of the hollowed circular material. Scale bars: A=25 micrometers; B=5 micrometers; C=0.5 micrometers.

FIG. 6. Scanning electron micrographs of *Staphylococcus aureus* cultivated 24 h on silk suture followed by overnight incubation with oxacillin 6.25 micrograms/mL. (A) Low-magnification view of suture incubated with the RN6390 strain showing clumps of ruffled matrix along and between the silk threads. (B) Strain 25923 showing cocci that appear relatively smooth (arrows) and others with more wrinkled cell walls decorated with fibrillar elements, seen more clearly at higher magnification (C). Scale bars: A=15 micrometers; B=36 micrometers; C=0.5 micrometers.

FIG. 7. Scanning electron micrographs of *Staphylococcus aureus* 25923 cultivated 24 h on silk suture followed by overnight incubation with vancomycin 50 micrograms/mL (**A**, **B**) or 100 micrograms/mL (**C**, **D**). (**A**) Ruffled matrix containing cocci enmeshed in fibrillar elements, with inset (**B**) more clearly distinguishing thicker (arrows) and thinner (**C**) fibrils. Portion of the biofilm with staphylococci embedded in a more consolidated matrix, more clearly seen at higher magnification in inset (**D**). Scale bars: **A**, **C** = 3 micrometers; **B**, **D** = 1 micrometer.

question the ability of an antimicrobial agent to penetrate this barrier and interact with its bacterial target. Under specific circumstances, some antimicrobial agents such as hypochlorite [22] and aminoglycosides [4] may not diffuse readily across a biofilm, but fluorescent tracers (~ 400 MW) penetrate biofilms [26], as do most biocides and antibiotics [4,5]. However, to our knowledge, no experimental design has assessed penetration of the microenvironment immediately surrounding individual bacterial cells in the depths of the biofilm.

If antibiotic-resistant cells are a population surrounded by an impermeable matrix, these cells would have many of the hallmarks commonly associated with persister cells; i.e., production stochastically, avoidance of multi-drug killing, viability irrespective of the antibiotic concentration, avoidance of immune killing by the host, and inability to transfer antibiotic resistance to progeny [5,8,9,24,27]. A mathematical model of biofilm dynamics predicts that, even though persister cells are unable to grow, these cells increase in number [27]. Although it is unclear if attachment to a substratum initiates EPS formation, this likely is the case. The dense EPS seen with SEM was focal and might be produced within specific areas in the biofilm, where perhaps surface contact (or other factors) triggers production of a specialized EPS. This idea not only is consistent with the common hallmarks of persister cells, but also would explain why no one has yet been able to isolate persister cells directly from an intact biofilm. Absolute proof that residual populations of resistant cells; i.e., persister cells, are those surrounded by dense ma-

trix would be a daunting task, but clarification of this concept is important.

There is a consensus that no single mechanism is likely to explain the greater antimicrobial resistance of biofilms [7]. In addition to putative persister cells that are inherently antibiotic resistant, other mechanisms responsible for resistance are postulated to include reduced bacterial growth rate, increased expression of resistance genes, decreased antibiotic penetration of biofilm EPS (via biochemical methods or physical walling off), and bacterial expression of specific factors such as multidrug efflux pumps [1–9,22–24,26–28]. The pharmaceutical industry is developing antibiotics that target the bacterial cell (membrane, DNA, specific proteins). In view of the results of the present study, perhaps greater effort should be directed to designing agents that penetrate the EPS, agents that might be used in conjunction with antibiotics to which the bacterial cell remains intrinsically susceptible.

Conclusion

Using three cell wall-active antimicrobial agents, the susceptibilities of *S. aureus* tested as planktonic cells were similar to the susceptibilities of mechanically dispersed biofilm cells cultivated on surgical suture. When suture-associated *S. aureus* biofilms were incubated with drug concentrations that were bactericidal for planktonic cells, a stable population of biofilm bacteria remained viable. Scanning electron micrographs of suture-associated biofilms showed that some staphylococci had smooth cells walls similar to planktonic

cells, whereas others were encased in extracellular material. Compared with control biofilms, this extracellular material appeared more abundant in antibiotic-treated biofilms and may be one factor inhibiting antibiotic binding to bacterial targets.

Author Disclosure Statement

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