

Adhesion of meticillin-resistant *Staphylococcus aureus* to DACC-coated dressings

- **Objective:** The aim of this *in vitro* study was to demonstrate the binding capacity of multiple meticillin-resistant *Staphylococcus aureus* (MRSA) strains and compare the binding capacity to meticillin-sensitive *Staphylococcus aureus*.
- **Method:** The binding of *Staphylococcus aureus* to a surface was assessed by bioluminescent monitoring of the bacterial ATP levels. This assay can be used as an *in vitro* diagnostic model for bacteria binding in a critically colonised wound.
- **Results:** Eleven strains of *Staphylococcus aureus* were examined including MRSA, all of which efficiently and equally adhered to the dialkyl carbamoyl chloride (DACC)-coated dressing (Sorbact; Abigo Medical AB). The binding capacity was all in the same range $0.7\text{--}2.9 \times 10^6$ CFU/cm², regardless of the antibiotic resistance properties of the specific strain.
- **Conclusion:** The decrease of wound bioburden of *Staphylococcus aureus* including MRSA is the result of the high binding capacity shown in this study and by earlier data. The findings in this study strengthen the held view that development of antibiotic resistance has minimal impact on the surface structures of the microorganisms in wounds.
- **Declaration of interest:** This work was supported by Abigo Medical AB, Sweden. The laboratory facilities for the MRSA work were provided by Sahlgrenska University Hospital and the Department for Clinical Bacteriology, University of Gothenburg, Sweden. No animals or humans were used in this study.

wound healing; adhesion; bacteria binding; *in vitro*; MRSA

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Meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *Staphylococcus aureus* (MRSA) cause similar infections, ranging from minor infections of the skin to major wound infections.¹ Individual immunity, size, localisation and the amount, and the virulence of the microorganisms present determine whether a wound remains harmlessly colonised or leads to infection.² MRSA is especially troublesome in hospitals, prisons and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of infection than the general public. Almost half of infections in burn patients at health facilities are caused by *Staphylococcus aureus*.³⁻⁶

An *in vitro* study conducted over 20 years ago enabled measurement of bacterial cell surface hydrophobicity (CSH) to assess the specific surface characteristics of microorganisms and their respective contribution to binding.⁷ This research group, led by Wadstrom and Ljungh, demonstrated the presence of cell surface hydrophobins influencing the ability of bacteria to colonise and bind to target. A wound dressing was designed where the microorganisms bound preferentially to the dressing rather than interacting with the wound.^{8,9}

The dressing used as a surface for adhesion in this

study consists of cellulose acetate-based fabric coated with dialkyl carbamoyl chloride (DACC). As a result of the coating, the dressing is afforded microbial binding properties. Microbial binding is achieved by non-specific factors such as physical hydrophobic interaction and specific adhesin factors including fibrinogen-binding protein.^{10,11} Binding is a complex process that involves the surface properties of microorganisms, wound and dressing. The initial process is normally non-specific, involving physical forces as hydrophobic interaction, electrostatic interaction, van der Waals forces, gravitational or Brownian motion.¹¹ The secondary process involves chemical interaction, where specific adhesion becomes predominant and involves specific molecular reactions between the bacterial surface structure and substratum surfaces (DACC-dressing). The benefit of a dressing of this type is to enhance control of the wound bioburden, reducing the overall demand placed on antibiotics, without using antimicrobial substances.

The aim of this *in vitro* study was to demonstrate comparable binding of clinical *Staphylococcus aureus* strains relative to multiple MRSA strains using a DACC-coated dressing as adhesion material. The study focuses on the adhesion as a virulence factor, which should not be confused with encapsulation, absorption, or killing of microorganisms.

Table 1. Characteristics of *Staphylococcus aureus* organisms

Target organism CCUG number	MRSA	Spa type, PFGE* type	comment
1. 2354	-	n.a	NCTC 7428, pure β toxin
2. 35603	+	n.a	QC MRSA, 1992, wound
3. 43063	+	t2363,F	1999, wound on blisters
4. 44509	+	SP	2000, wound from groin
5. 45435	+	t012, SM	2001, wound on the mouth
6. 46315	+	t044, P	2002, wound on the knee
7. 47418	+	t067, J	2003, wound on the toe
8. 53315	+	t008, K	2006, wound on the thigh
9. 56450	-	n.a	2008, wound on the elbow
10. 58494	+	t018, SP	2009, wound from heel
11. 60578	+	t044, P	2010, unspecified wound secretion

*Pulsed-field gel electrophoresis: n.a: Neither Spa type nor PFGE type available, QC=quality control

Method

Materials

The dressings used in the study consisted of a cellulose acetate-based fabric coated with DACC (Sorbact Compress, Abigo Medical AB). The same uncoated cellulose-acetate fabric was used as a control. Both the uncoated and coated dressings were sterile.

Eleven isolates were used in this study. The MRSA strains were isolated from different inpatient and outpatient wounds (Table 1) and all the strains were tested for presence or absence of Nuc-gen and MecA-gen using real-time PCR. The isolate from culture collection at University of Gothenburg, CCUG 35603, was used as control for MRSA. The strains CCUG 2354 and CCUG 56450 were used as isolates for meticillin-sensitive strains. The bacterial strains

were chosen were isolated from different wounds, and had different pulsed-field gel electrophoresis (PFGE) and Spa type. The target organisms, origin are presented in table 1. All the MRSA isolates in this study are clinical and have caused infection in patients who sought treatment.

Storage and preparation of bacteria

Cell material from each strain was stored in Hogness freezing medium at -80°C . The bacterium were grown on 5% horse-blood agar plates for 48 hours before harvesting. Bacteria were further grown on horse-blood agar plates for an additional 18 hours to be used for adhesion experiments.

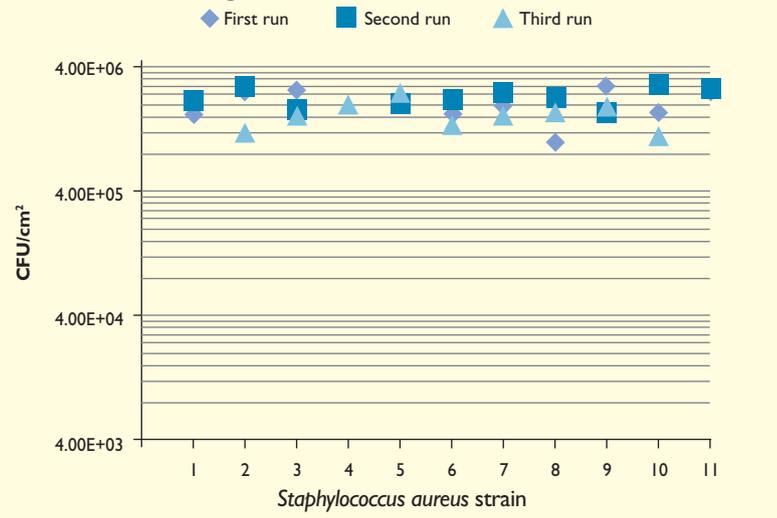
Bacterial cells were washed and centrifuged for 15 minutes (2500g, 20°C). The supernatant was discarded and the cells were re-suspended (phosphate buffer, 0.02M, pH 6.8). The turbidity of the suspension was measured with a spectrophotometer at $\lambda=610\text{nm}$ and the concentration of bacterial suspensions were subsequently adjusted to 1×10^8 CFU/ml in each of the eleven cell suspensions. The exact concentration was obtained through plating results the day after the luminometer run. The cells were grown on blood agar plates after serial dilutions and incubated at 37°C overnight. The viable number of bacteria (CFU/ml) was determined by colony counts on plates consisting of 30–300 colony-forming units.

Firefly assay of adenosine 5'-triphosphate (ATP) for adhesion

To study the binding of bacteria to a dressing surface, an inoculum of each strain was added to the wound dressing. The bioluminescence technique was used to quantify the bacterial adenosine 5'-triphosphate (ATP) levels against a reference standard curve. The enzyme in the ATP kit (Sigma-Aldrich, Sweden) reacts with the ATP in live bacteria, resulting in light emission.

Fresh bacterial suspensions were prepared and kept on ice for no longer than one hour before the assay was performed. Punched samples of fabric with a 1cm^2 single layer of the coated dressing were incubated with $250\mu\text{l}$ of 1×10^8 CFU/ml bacterial suspension at 20°C for one hour, while shaking at 100rpm. After adhesion, the dressing was rinsed three times with 0.02M phosphate buffered saline (pH6.8) to remove loosely adhered bacterial cells. The numbers of bacterial cells adhering to the dressing were determined by a standardised luminescence technique for ATP detection.¹² The kit (Sigma Aldrich, Sweden) contained a luciferase enzyme which is dissolved in 5ml of sterile water to generate a stock solution. From the stock solution, $200\mu\text{l}$ was added to untreated, flat-welled, white ATP-free 96-well polystyrene microtiter plates (Costar 3912, Corning, Sweden). The light emitted from bacterial

Fig 1. Adhesion of *Staphylococcus aureus* including MRSA to DACC-coated dressing



ATP was measured at 20°C with a luminometer (Lumistar Omega, LabVision, Sweden). The ATP reagents and samples were kept on ice during preparation. Sterile buffer was used as negative control. Unless stated each of the bioluminescence assays was run with two different samples of the strain of bacteria on three separate occasions.

Statistics

Comparison of CFU/cm² was performed using Fisher's exact test (GraphPad Prism; GraphPad Software, La Jolla, CA). A two-tailed p-value of 0.05 was defined as statistically significant.

Results

Adhesion to the DACC-coated dressing of the *Staphylococcus aureus* control strain CCUG 2354, clinical strain CCUG 56450 and nine MRSA strains in colony-forming units per dressing sample (CFU/cm²) are presented in table 2 and fig 1. There were two samples of each of the 11 bacterial strains in each run. The data in table 2 shows both sample values separated by a semicolon. The initial binding capacity demonstrated for *Staphylococcus aureus* including MRSA in the experiments was 0.7–2.9x10⁶ CFU/cm². These experiments were repeated three times as shown in table 2.

A comparison of the adhesion capacity between DACC-coated dressing material and uncoated control with the *Staphylococcus aureus* control strain CCUG 2354 with an initial concentration of 8x10⁷ CFU/ml was performed. Three parallel samples, were used for coated and uncoated control (Fig 2). The DACC-coated material bound a mean value of 1.5x10⁶ CFU/cm², whereas uncoated binding was reduced to a mean value of 6.8x10⁴ CFU/cm², which was significantly lower (p<0.0001; Fisher's exact test).

To illustrate the mode of action, binding of a mixed culture containing *Staphylococcus aureus* control strain (CCUG 2354), *Pseudomonas aeruginosa* (CCUG 17919) and *Candida albicans* (CCUG 32723) to the DACC-coated dressing was examined at high resolution (Fig 3). The figure shows not only binding to the material, but also aggregation between microbes. The electromicrograph was produced externally (TATAA Biocenter AB, Gothenburg, Sweden).

Discussion

The results of this study are intended to promote clinical confirmation of reducing the MRSA impact in the wound care setting. Due to the complications associated with antibiotics, there is a need for non-antibiotic management strategies as an alternative or combined therapy when absolutely necessary.

The *Staphylococcus aureus* isolates used in our adhesion experiments were collected from different wound environments and analysed by Spa typ-

Table 2. Adhesion of *Staphylococcus aureus* including MRSA to DACC-coated dressing

Target organism CCUG no	Run 1	Run 2	Run 3
	× 10 ⁶ CFU/cm ²	× 10 ⁶ CFU/cm ²	× 10 ⁶ CFU/cm ²
1. 2354	2.1; 2.2	2.0; 2.4	1.5; 1.8
2. 35603	1.1; 1.2	2.8; 2.9	2.5; 2.7
3. 43063	1.6; 1.7	1.8; 1.9	2.3; 2.8
4. 44509	1.9; 2.1	n.a	n.a
5. 45435	2.4; 2.6	1.9; 2.2	2.3; 2.4
6. 46315	1.3; 1.5	1.9; 2.5	1.7; 1.7
7. 47418	1.6; 1.7	2.2; 2.8	1.5; 2.4
8. 53315	1.7; 1.8	2.1; 2.5	0.7; 1.2
9. 56450	1.9; 2.0	1.6; 1.9	2.8; 2.9
10. 58494	1.1; 1.2	2.7; 3.2	1.7; 1.8
11. 60578	n.a	2.7; 2.8	2.6; 2.7

n.a: not available

ing and PFGE. The expression characteristics of the strains used could indicate different surface properties, resulting in the possibility of different adhesion capacity. Furthermore it is likely that the meticillin-sensitive *Staphylococcus aureus* and MRSA strains have different adhesins on their cell walls. All of these features, together with morphological structures, can be involved in facilitating adhesion to the DACC-coated dressing. The bacteria can also bind to the target through various receptors, to further increase the binding to the dressing material. However, recent studies of the anti-infectious agents against MRSA imply the cell surface structures con-

Fig 2. Adhesion of *Staphylococcus aureus* to DACC-coated dressing material (n=3) and uncoated control (n=3).

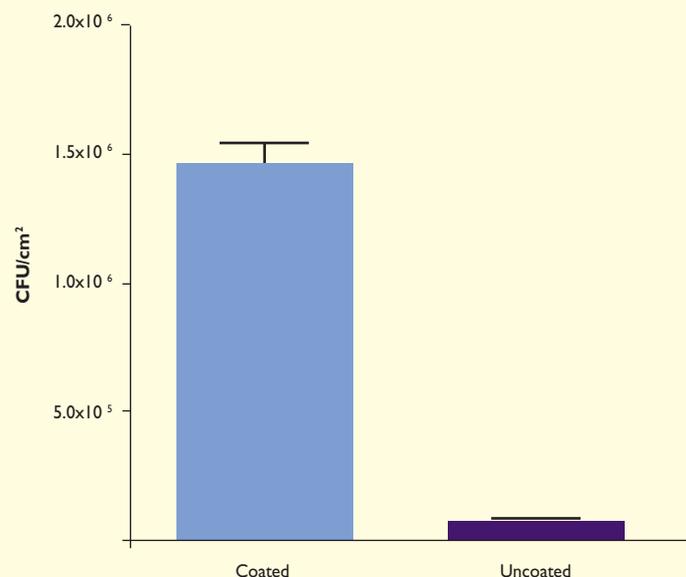
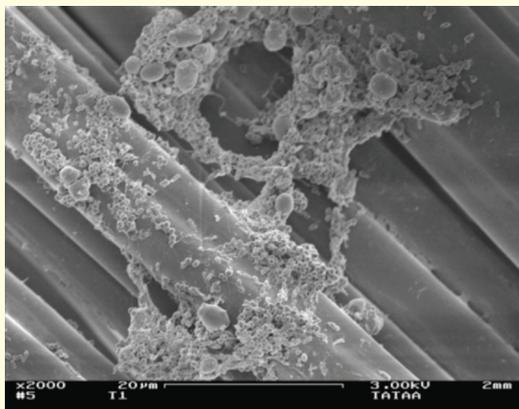


Fig 3. Adhesion of microorganisms including *Staphylococcus aureus* to DACC dressing



sisting of hydrophobins for both methicillin-sensitive *Staphylococcus aureus* and MRSA strains are similar.¹³⁻¹⁵ Our binding experiments showed that the DACC-coated dressing material bound all the strains with a similar affinity, indicating that the physicochemical forces dominate over these specific chemical-binding interactions.

The binding capacity of the wound pathogens to the DACC-coated dressing demonstrated here is in line with other *in vitro* data obtained by Ljungh and coworkers.⁹ We also confirm the results of the

recent data showing that the microbial binding capacity of the uncoated material compared with that of the DACC-coated dressing and was significantly lower.¹⁶ Although these studies are not evidence of a clinical effect, we anticipate the results demonstrated may improve the understanding of the clinical research and management paradigms for local wound care.

Further research in this area could include assessing the CSH of the different MRSA strains, as well as comparing a control dressing with a bacterial binding dressing on wounds to quantify the efficacy in a clinical setting. In the future, it may be possible to develop wound dressing material targeting a specific wound pathogen, where chemical binding will be the dominant force.

Conclusions

A quantitatively high and stable initial adhesion to the microbial binding dressing was detected in all experiments involving *Staphylococcus aureus* strains including the nine MRSA strains. These findings strengthen the view that development of antibiotic resistance has minimal impact on the surface structures of the microorganism from a wound adhesion perspective. In this case, the surface structures of non-antibiotic resistant *Staphylococcus aureus* are present in similar composition, as on the MRSA strains. ■

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