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MAYO CLINIC

# Locally Delivered Antistaphylococcal Lysins Exebacase or CF-296 is Active in Methicillin-Resistant *Staphylococcus aureus* Implant-associated Osteomyelitis

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## ABSTRACT

**Background:** *Staphylococcus aureus* orthopedic infections often require surgery and long-term antibiotics; better therapeutics are needed. The antistaphylococcal lysins exebacase (EXE) and CF-296 have rapid bactericidal activity and a low propensity for development of resistance, and exhibit synergy with antibiotics. Using a rabbit model of implant-associated osteomyelitis (IAO), we tested the activity of EXE and CF-296 delivered locally, with and without daptomycin (DAP).

**Methods:** The rabbit IAO infection was induced by drilling a hole into the medial tibia and irrigating the medullary canal with water, followed by 0.6 ml of local treatment with EXE (10.64 mg/ml), CF-296 (10.16 mg/ml) or lysin carrier. A colonized titanium screw with MRSA IDRL-6169 (minimum inhibitory concentration of DAP, EXE, and CF-296, 0.5 µg/ml) was inserted. Intravenous DAP (6 mg/kg) or saline was delivered and continued daily. On the fifth day, rabbits were euthanized, and tibiae and implants collected for culture. Results were reported as $\log_{10}$ cfu/g of bone or per implant and comparisons among the six groups performed using the Wilcoxon rank sum test.

**Results:** All treatments had activity compared to the control group. Compared to DAP, there were 1.15, 1.46, and 1.70 $\log_{10}$ cfu reductions in bones receiving EXE, EXE/DAP, and CF-296/DAP, respectively. MRSA on implants was reduced by 3.87, 3.48 and 3.17 $\log_{10}$ cfu for EXE, EXE/DAP, CF-296/DAP, respectively, compared to DAP. EXE alone resulted in greater cfu reductions on implants than CF-296 alone; there was no difference between the two lysins' activity when delivered locally in conjunction with DAP for bone or implant cultures.

**Conclusions:** Lysins, administered locally in addition to traditional therapies, may offer a potential strategy for combatting *S. aureus* implant-associated infections.

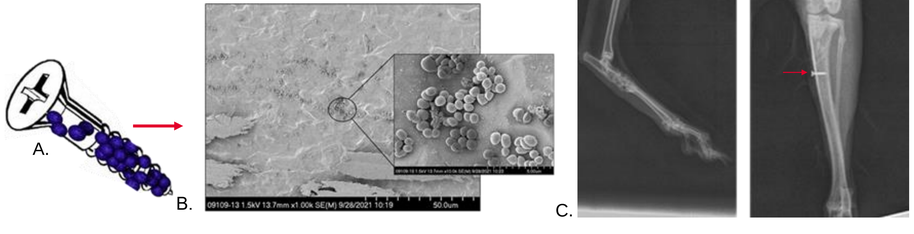


**Figure 1.** Screw colonized with MRSA (A), SEM of MRSA on a screw (B) and an X-ray of implanted screw into a rabbit tibia (C).



## BACKGROUND

### *Implant Associated Osteomyelitis*[1,2]

- *Staphylococcus aureus* most common cause
- Intracellular invasion and biofilm formation → evades antimicrobials and immune system
- Surgical intervention and long-term antibiotics
- Unmet need for new antimicrobial strategies
- Local antimicrobial delivery - potential solution

### *Antistaphylococcal lysins*

- Exebacase (CF-301) and CF-296
- Hydrolytic enzymes (native or engineered) from bacteriophage[3,4]
- Rapidly bactericidal[3,4]
- Active against biofilms[3,4]
- Active (delivered systemically) in rat osteomyelitis model (high bone concentrations)[5,6]

### *Study Objective*

- Develop rabbit implant associated MRSA osteomyelitis model (Figure 1)
- Treat infection locally with lysins ± systemic daptomycin (Table 1)

## METHODS

### *Implant Colonization*

- 1.5x7 mm titanium cortex screws (DePuy Synthes, Monument, CO)
- 37 µl $10^4$ cfu/ml MRSA IDRL-6169 + screw @ 37C for 16 hours

### *Animal Model*

- 2-3 kg male & female New Zealand rabbits (Figure 2)
- Insert colonized screw (~$10^5$ CFU/screw) into left tibia (Figure 1C)
- Surgical procedure (Figure 3)
- Treatment Groups (Table 1)

**Table 1.** Treatment groups

| N=10/group | Systemic (6 mg/kg, IV, daily x4) | Local (0.6 ml, single dose, intramedullary) |
|---|---|---|
| Group 1 | Saline | Carrier |
| Group 2 | Daptomycin | Carrier |
| Group 3 | Saline | Exebacase |
| Group 4 | Daptomycin | Exebacase |
| Group 5 | Saline | CF-296 |
| Group 6 | Daptomycin | CF-296 |

**Figure 2.** New Zealand rabbit (Envigo, Indianapolis, IN)

### *Bone Collection and Culture*

- Day 5, rabbit euthanized, tibia and screw collected for quantitative culture
- Tibia cryopulverized @ 28 Hz for 90 seconds with Mixer Mill 400 (Retsch, Newtown, PA) using 50 ml stainless steel grinding
- Screw & bones vortexed/sonicated (40 kHz, 0.22 w/cm$^2$), ten-fold dilutions prepared, plated on blood agar plates, and incubated 24 hours in 5% $CO_2$ at 37C
- Results reports $\log_{10}$ cfu/g of bone or per implant and comparisons performed using the Wilcoxon rank sum test

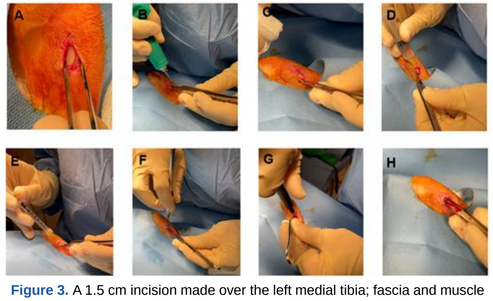


**Figure 3.** A 1.5 cm incision made over the left medial tibia; fascia and muscle cleared to expose smooth flat portion of tibia (A). Using a micro drill, a hole created through cortical bone into medullary cavity (B and C). 2 ml water (D) followed by 0.6 ml lysin or lysin carrier (E) injected into hole. Seeded implant (~5.24 $\log_{10}$ cfu/implant) inserted into hole (F). Screw tightened with screwdriver (G) and implant confirmed to be secure (H).

## RESULTS (FIGURE 4)

- All treatments active compared to saline/carrier group (P≤0.0025)
  - >3 $\log_{10}$ cfu reductions with exebacase, exebacase with daptomycin, CF-296 with daptomycin
- Compared to daptomycin alone, 3.87 (P=0.007), 3.48 (P=0.0015) and 3.17 (P=0.0064) $\log_{10}$ cfu reductions on implants in animals receiving exebacase, exebacase with daptomycin, and CF-296 with daptomycin, respectively
- Locally delivered exebacase alone → greater cfu reductions on implants than locally delivered CF-296 alone (P=0.0015)
- No difference between two lysins' activity when delivered locally in conjunction with systemic daptomycin, based on bone or implant cultures

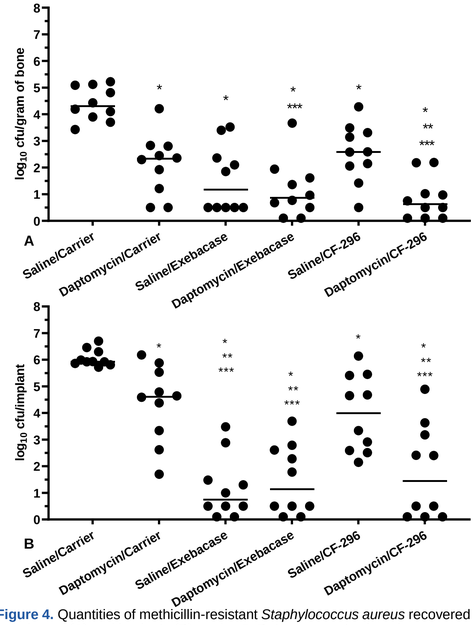


**Figure 4.** Quantities of methicillin-resistant *Staphylococcus aureus* recovered from bones (A) and implants (B) on day 5. Dots represent values from individual animals; horizontal lines represent median values. Asterisks indicate significant reductions compared to *saline/carrier (P≤0.0025), **daptomycin/carrier (P≤0.0098), or ***saline/CF-296 (P≤0.0154).

## DISCUSSION

- Applying treatment locally to affected area allows immediate, direct contact with infection, compared to delayed delivery and variable low concentrations of available drug in bone with systemic dosing
- Results indicate that locally delivered lysins reduce bacterial loads *in vivo*
- >3 $\log_{10}$ reduction in bacterial counts, in both implant and bone cultures, with exebacase alone and both lysin with daptomycin *versus* controls as well as in implant cultures *versus* daptomycin alone

## CONCLUSIONS

- All treatment groups had significantly reduced amounts of MRSA recovered from bone and implants
- Exebacase, exebacase with daptomycin and CF-296 with daptomycin were most active, with significant reductions of MRSA counts compared to daptomycin alone on the implants
- No difference between the efficacy of the two lysins delivered locally when administered with daptomycin
- Lysins, administered locally in addition to traditional therapies, may offer potential strategy for combatting *S. aureus* implant-associated infection

## REFERENCES

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