SCV phenotype and reduced intracellular activity of antibiotics: a cause for persistent staphylococcal infection?

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SCVs of *S. aureus* as a cause of persistent foreign body infection


Prosthetic valve endocarditis due to small-colony staphylococcal variants.

Raddour LM, Christensen GD.


Bloodstream infections caused by small-colony variants of coagulase-negative staphylococci following pacemaker implantation.


Small colony variants of *Staphylococcus aureus* and pacemaker-related infection.


Emerging *Staphylococcus* species as new pathogens in implant infections.

von Eiff C, Arciola CR, Montanaro L, Becker K, Campoccia D.


Small-colony variants (SCVs) of staphylococci: a role in foreign body-associated infections.

von Eiff C, Becker K.
SCV : case under study

- SCV isolated from a patient
  - with complicated prosthetic vascular graft infection and bacteraemia,
  - unsuccessfully treated successively with
    - cotrimoxazole (SMX/TMP),
    - minocycline (MIN),
    - a combination of vancomycin and rifampin (VAN-RIF)
    - a combination of linezolid and rifampin (LNZ-RIF)

- thymidine-auxotrophic MRSA, growing as tiny, non-pigmented and non-hemolytic colonies on Columbia blood agar.

- resistant to OXA, SXT, CLI, LIN, ERY, quinupristin and TET.
Aim of the study

To examine the extracellular and intracellular activity against this SCV of:

- Antibiotics unsuccessfully used to treat the patient
  - cotrimoxazole (SMX/TMP)
  - minocycline (MIN)
  - vancomycin + rifampin (VAN-RIF)
  - linezolid + rifampin (LNZ-RIF)

- Other approved antistaphylococcal antibiotics,
  - gentamicin (GEN)
  - moxifloxacin (MXF)
  - quinupristin-dalfopristin (Q-D)
  - tigecycline (TGC)
  - daptomycin (DAP)

- Antibiotics in the late stages of development: lipoglycopeptides
  - telavancin (TLV)
  - oritavancin (ORI)
Growth of SCV in broth and within macrophages

![Graph showing growth of SCV in broth and within macrophages.](image_url)
Subcellular localization of SCV

THP-1 macrophages

HUVEC endothelial cells

SCV

Lysotracker

merged
Extracellular activity

SCV 397 extracellular activity at Cmax

AB received by the patient

Δ log CFU from initial inoculum

limit of detection

-5 -4 -3 -2 -1 0 1 2 3

5 h 24 h

Ctr SXT MIN VAN LZD RIF GEN MXF Q-D DAP TGC TLV ORI
Intracellular activity in THP-1 macrophages

SCV 397 intracellular activity in THP-1 at Cmax

AB received by the patient

Δ log CFU from initial inoculum

Ctr  SXT  MIN  VAN  LZD  RIF  GEN  MXF  Q-D  DAP  TGC  TLV  ORI

5 h
24 h
Intracellular activity in THP-1 macrophages

SCV 397 intracellular activity in THP-1 at Cmax

Δ log CFU from initial inoculum

-3 -2 -1 0 1 2

5 h 24 h 72 h

AB received by the patient

Ctr SXT MIN VAN LZD RIF GEN MXF Q-D DAP TGC TLV ORI

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Intracellular activity in HUVEC endothelial cells

SCV 397 intracellular activity in HUVEC at Cmax

AB received by the patient

Δ log CFU from initial inoculum

5 h
24 h

Ctr  SXT  MIN  VAN  LZD  RIF  GEN  MXF  Q-D  DAP  TGC  TLV  ORI

06-11-2008  SBIMC- 2008
Intracellular activity in HUVEC endothelial cells

SCV 397 intracellular activity in HUVEC at Cmax

$\Delta \log$ CFU from initial inoculum

-4 -3 -2 -1 0 1 2 3

Ctr SXT MIN VAN LZD RIF GEN MXF Q-D DAP TGC TLV ORI

5 h 24 h 72 h

AB received by the patient
Dose-effect at 24 h

antibiotics received by the patient

Minocycline

MIC = 4

Vancomycin

MIC = 2

Linezolid

MIC = 0.002

Rifampin

MIC = 1

Oritavancin

MIC = 0.125

most active drug
Combinations at 24 h

combinations received by the patient

combination at Cstatic

Δ log from initial inoculum

Time (h)
Combinations at 24 h

combinations received by the patient

![Graph showing combinations at Cstatic over time]

- LNZ
- RIF
- VAN
- RIF-LNZ
- RIF-VAN

Time (h)

Δ log from initial inoculum

0 6 12 18 24
Combinations at 24 h

combinations received by the patient

combination at Cstatic

combination at Cmax
Combinations at 24 h

combinations received by the patient

combination at C_{static}

Time (h)

$\Delta \log$ from initial inoculum

combination at C_{max}

Time (h)

$\Delta \log$ from initial inoculum
Combinations at 24 h

combinations between more active drugs

combination at Cmax

$\Delta \log$ from initial inoculum

Time (h)
Combinations at 24 h

combinations between more active drugs

![Graph showing combinations at Cmax]

- Δ log from initial inoculum
- Time (h)
- Combination at Cmax
- Lines representing different drug combinations:
  - MXF
  - MXF-RIF
  - MXF-ORI
  - ORI
  - RIF
Combinations at 24 h

combinations between more active drugs

Combination at Cmax

Δ log from initial inoculum vs. Time (h)

- MXF
- MXF-ORI
- MXF-RIF
- ORI
- RIF
- RIF-ORI
Conclusions

- SCV can persist intracellularly for prolonged periods of time; they remain totally (THP-1) or mainly (HUVEC) confined in acidic vacuoles.

- All antibiotics were considerably less active intracellularly than extracellularly against SCVs.

- As anticipated for thymidine-dependent SCVs, SMX-TMP was ineffective against both extracellular and intracellular forms. The use of this drug may have contributed to select SCV phenotype upon treatment.

- None of the antibiotics administered to the patient reached a bactericidal effect against intracellular SCVs. Activity of RIF was decreased in combination with LZD or VAN. This may explain the observed failure of antibiotic treatment in this patient and the difficulty of eradicating these organisms in general.

- In infected cells, ORI was the most active drug, and this activity was further improved when combined with RIF.

- Our cellular model may serve to evaluate antibiotic susceptibility in infections for which intracellular reservoirs and SCVs could play an important role.