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# Open wounds and rifampicin therapy are associated with rifampicin resistance among staphylococcal vascular graft/endograft infections

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**Background:** Optimal timing for rifampicin combination therapy in patients with staphylococcal vascular graft/endograft infection (S-VGEI) is unknown. Experts recommend adding rifampicin after lowering bacterial load by surgery and wound closure.

**Objectives:** To assess predictors of rifampicin resistance among staphylococci isolated from patients in the Vascular Graft Infection Cohort Study.

**Methods:** We included prospective patients with S-VGEI diagnosis from 1 January 2002 to 30 June 2020. We retrospectively assessed determinants of rifampicin resistance using exact logistic regression and described survival with Kaplan–Meier curves.

**Results:** We analysed 513 *Staphylococcus* spp. among 143 predominantly male (82%) patients with a median age of 68 years (IQR 60–75). Thereof, 82 (57%) received a rifampicin combination therapy and 61 (43%) received an antimicrobial therapy without rifampicin. Among 82 patients with rifampicin, 26/26 patients with any rifampicin resistance had open wounds with a strong association of rifampicin resistance with rifampicin treatment while having open wounds (OR 37, 95% CI 6.1 to $\infty$). Among 75 patients with a rifampicin combination therapy and rifampicin-susceptible staphylococci at S-VGEI diagnosis, 12/12 patients with a secondary rifampicin-resistant isolate had an open wound (OR 14, 95% CI 2.1 to $\infty$).

**Conclusions:** Rifampicin should be started after wound closure due to increased risk of rifampicin resistance observed while having open wounds or second-look surgeries among patients with S-VGEI.

## Introduction

Vascular graft/endograft infections (VGEI) are feared complications among patients with vascular grafts.[1–3] Standard treatment of VGEI consists of surgical treatment with total or partial graft excision and debridement combined with pathogen-specific antimicrobial therapy.[1,2] Some experts also recommend graft-preserving approaches with negative pressure wound therapy (NPWT).[2,4]

VGEI are biofilm-associated infections. Thus, staphylococci isolated from chronic infections may be in a dormant, stationary phase, tolerate antibiotic challenges and be capable of resuming growth.[5] Accordingly, antimicrobial treatment of VGEI due to *Staphylococcus aureus* or coagulase-negative staphylococci (S-VGEI) is given over a prolonged time period with the intention of killing the biofilm-associated bacteria.[1,6] From the existing literature, drug combination therapies are preferred to monotherapy for S-VGEI.[1,6,7] First-line treatments for methicillin-susceptible S-VGEI are methicillin derivatives together with an aminoglycoside and for methicillin-resistant S-VGEI the combination of a glycopeptide together with an aminoglycoside.[6] Whenever the strain is susceptible, rifampicin should be prescribed in foreign body-associated

infections due to its bactericidal activity on staphylococcal biofilms.[6,8,9] Of note, the optimal starting time for rifampicin therapy in patients with S-VGEI is not well-defined.[10] Rifampicin resistance might occur if there is a larger burden of bacilli, if there are inadequate companion drugs to suppress growth of rifampicin-resistant clones or if rifampicin-resistant staphylococci of the skin microbiota (via selection pressure on the local flora) enter the surgical site via second-look surgeries, open wounds or indwelling drainages.[8,9] Accordingly, some experts recommend adding rifampicin only after the bacterial load is lowered by surgical treatment, removal of drains or cessation of bacteraemia.[9,11,12] However, the basis of this recommendation is tenuous and there is a lack of data in the setting of S-VGEI.

We determined associations of ongoing rifampicin treatment during open wounds, indwelling drainages and bacteraemia on primary and secondary rifampicin resistance in staphylococci isolated from patients with S-VGEI. Moreover, we were interested in the associations of rifampicin resistance on cure and survival among patients with S-VGEI.

## Methods

### *Study design and study population*

VASGRA is an ongoing prospective, observational cohort study with continued enrolment of patients aged 18 years or older receiving a vascular graft operation at the University Hospital of Zurich, Switzerland since April 2013.[13] The study protocol was approved by the Institutional Ethics Committee in Zurich, Switzerland (KEK-ZH-2012-0583; PB 2016-01320). VGEI cases from 1 January 2002 to 30 March 30 2013—and hence occurring before the formal establishment of the VASGRA cohort—were also collected (KEK-ZH-2013-0179). After combining the two patient collections with VGEI, we limited the analysis to patients with detection of staphylococci (Figure S1, available as Supplementary data at *JAC* Online). A baseline and a follow-up visit were required for inclusion in the study.

### *Variables*

Every case was adjudicated using the Management of Aortic Graft Infection Collaboration criteria.[14] Information on S-VGEI included age, sex, BMI, location of infection (abdominal, thoracic, peripheral); type of graft material [polytetrafluorethylene (PTFE); polyethylene terephtalate (PET), Dacron®]; biological graft or other material, type of antimicrobial therapy and the surgical treatment for VGEI (debridement with partial excision of graft; debridement with total graft excision or graft replacement; no surgery; debridement/retention of graft with/without NPWT). We documented potential drains or open wounds in the context of vascular or cardiac surgeries. According to our in-house treatment algorithm, we used NPWT treatment to promote wound healing and remove exudate in acute and chronic wounds and VGEI with involvement of the body of the graft and intact graft-vessel anastomosis.[2,4]

We collected information on all bacterial isolates containing *S. aureus* or CoNS at baseline (diagnosis of S-VGEI) and over time until VGEI cure had occurred, end of follow-up or death. We rated the importance of the respective isolates and collected information on blood and tissue cultures, biopsies, and bacterial isolates retrieved from vascular grafts and rated the importance of the respective isolates. CoNS (as potential contaminants) were rated as relevant pathogens if at least two intraoperative specimens or blood cultures, or at least one intraoperative specimen and one blood culture, were positive.[15] We did not consider other bacteria, which may have followed after the two species disappeared.

All staphylococci were processed at the Institute of Medical Microbiology, University of Zurich, Switzerland according to international standards.[16] Antimicrobial susceptibility testing was performed by Etest according to EUCAST guidelines.[17,18] Susceptibility of the causative bacterium to rifampicin was assessed at baseline and follow-up.

### *Definitions*

We used the term 'open wound' in case of: (i) dehisced, exuding surgical wounds; (ii) open abdomen treatment after laparotomy; (iii) open chest treatment in cardiac surgery; (iv) second-look surgeries; and (v) NPWT.

In most S-VGEI, a 6 week course of parenteral antimicrobial therapy was followed by an oral regimen for a median of 10 months of therapy. Monotherapy contained either anti-staphylococcal cell-wall active agents such as β-lactams or glycopeptides/lipopeptides. Combination therapy was defined if two anti-staphylococcal antibiotics were used at the same time (other combination therapy) or if anti-staphylococcal antibiotics were used together with rifampicin (combination therapy; standard dose of rifampicin $2 \times 450$ mg per day). These definitions referred to intravenous and oral treatment. In case of a mixed consecutive policy, we chose the type of therapy that was administered for the majority of time (>75%). We differentiated three situations: (i) any rifampicin resistance (any documented rifampicin resistance irrespective if primary or secondary); (ii) primary rifampicin resistance (rifampicin resistance before start of antimicrobial treatment); and (iii) secondary rifampicin resistance rifampicin (resistance emerging during antimicrobial treatment).

Preconditions for cure of S-VGEI were: (i) no new revision surgery; or (ii) no local or systemic signs of infection connected to the original S-VGEI site. We used the timepoint of stop of antimicrobial therapy as a proxy for the date of cure from S-VGEI (but only if >1 month before death). We documented all reasons for deaths among participants (death due to all causes; death due to S-VGEI).

### *Statistical analysis*

Patient- and procedure-related variables and antimicrobial treatment were assessed overall and if rifampicin combination therapy had been used or not. We used Fisher's exact and chi-square tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. We analysed factors associated with rifampicin resistance among staphylococci using exact logistic regression. We performed sensitivity analyses since we aimed to discern if the direction and the magnitude of the findings was the same in the prospective and retrospective cohort and if there were differences of CoNS in contrast to *S. aureus*. We calculated Kaplan–Meier survival curves for all patients and for the subset of patients with a susceptible *Staphylococcus* spp. at VGEI diagnosis depending on rifampicin treatment and drug resistance. Differences were compared using log rank tests. Stata/SE Version 16.1 (StataCorp, College Station, Texas, USA) was used for analyses.

## Results

From 1 January 2002 through 30 June 2020, 711 patients contributed to the analyses (Figure S1). We excluded participants not meeting the criteria for VGEI diagnosis ($n = 471$), with VGEIs due to other bacteria than staphylococci ($n = 93$), missing microbiological data ($n = 2$) or missing follow-up ($n = 2$),

### *Baseline characteristics*

Overall, 513 *Staphylococcus* spp. were isolated from patients (multiple isolates per patient were possible). Thereof, 78% were CoNS and 22% were *S. aureus*. Susceptibility to rifampicin was tested

among all isolates: one out 114 *S. aureus* isolates (0.9%) and 132/399 (33%) CoNS isolates were rifampicin-resistant either at VGEI diagnosis or during follow-up (Figure S2).

Clinical characteristics of participants and detection of staphylococci stratified by rifampicin treatment, are shown in Table 1. Overall, 143 patients were eligible for analysis, whereby 82 (57%) received a rifampicin combination therapy and 61 (43%) received an antimicrobial therapy without rifampicin (Table 1). The probability of rifampicin treatment was increased among VGEI of the thoracic aorta, whereas fewer patients received rifampicin combination therapy in case of a peripheral and abdominal VGEI. Patients with *S. aureus* were more likely to receive rifampicin than patients with CoNS (82% versus 58%). Reasons for non-treatment with rifampicin were: primary resistance (8.4%), pathogen rated as contaminant (19%), complete graft excision (6.3%), open wounds or drainages (8.4%), or bacteraemia (0.7%).

### *Predictors of rifampicin resistance*

Overall, rifampicin resistance emerged among 26% of patients within a median of 14 days (IQR 6–65) of antimicrobial therapy. Comparing the group with rifampicin combination therapy and the group without rifampicin, 26/82 (32%) and 12/61 (20%) patients had rifampicin-resistant staphylococci, respectively ($P = 0.07$). At the time of S-VGEI diagnosis, 7/26 patients in the rifampicin combination therapy group received rifampicin empirically before obtaining a laboratory report of rifampicin resistance, whilst 19 patients had a rifampicin-susceptible isolate at S-VGEI diagnosis and developed a secondary rifampicin resistance while on targeted rifampicin treatment. An open wound was reported among all patients with rifampicin resistance and rifampicin combination treatment, whereas 27/56 (48%) without rifampicin resistance had an open wound ($P < 0.001$). A surgical treatment by NPWT was present in 9/16 (65%) patients with rifampicin combination therapy and rifampicin-resistant staphylococci ($P = 0.02$).

Among the 75 patients with a rifampicin combination therapy and documented rifampicin-susceptible staphylococci at S-VGEI diagnosis, 12 (26%) developed a secondary rifampicin resistance in the course of the disease. All 12 patients had an open wound while receiving rifampicin treatment ($P = 0.001$), while only 34/63 (54%) patients without rifampicin resistance had an open wound. One patient with a secondary rifampicin resistance had an indwelling drain ($P = 0.66$).

Potential associations of rifampicin treatment and any rifampicin resistance (26 events among 82 patients) or secondary resistance (12 events among 75 patients) are shown in Figure 1. With an OR of 37 (95% CI 6.1 to $\infty$) and an OR of 14 (95% CI 2.1 to $\infty$) any rifampicin resistance and secondary rifampicin resistance were strongly associated with rifampicin treatment during open wounds, respectively. Neither indwelling drainages nor bacteraemia were associated with rifampicin resistance, possibly due to low numbers.

### *Sensitivity analyses*

The cohort variable did not change either the associations with open wound and any rifampicin resistance (OR 27, 95% CI 4.2 to $\infty$) or open wound and secondary rifampicin resistance (OR 12, 95% CI 1.8 to $\infty$). The associations also remained the same irrespective of type of staphylococci (CoNS or *S. aureus*) involved open wounds and any rifampicin resistance (OR 27, 95% CI 4.2 to $\infty$) and open wound and secondary rifampicin resistance (OR 10, 95% CI 1.5 to $\infty$).

### *Association of rifampicin treatment and secondary rifampicin resistance with survival and cure among patients with S-VGEI*

Median follow-up time of the cohort was 2.1 years (IQR 0.63–5.2). Overall survival of S-VGEI patients stratified by rifampicin treatment is shown in Figure 2(a). Out of 143 S-VGEI patients, 35 (24%) died after 1.42 years of follow-up per participant (203 years of follow-up overall). There was no evidence of an increased survival with rifampicin treatment ($P = 0.2$). Figure 2(b) shows survival among the 75 participants with susceptible staphylococci at S-VGEI diagnosis depending on whether a rifampicin resistance occurred or not. The total follow-up time was 114 years, with the observation time being censored at 2 years (average of 1.52 years of follow-up per patient). There was a trend of decreased survival among patients with secondary rifampicin-resistant staphylococci ($P = 0.09$).

Out of 143 patients, 121 (85%) stopped antibiotics and achieved cure of S-VGEI after a total follow-up time of 103 years. Rifampicin treatment was not associated with an improved healing rate ($P = 0.81$) (Figure S3A). Among the 75 patients with a rifampicin-susceptible isolate at baseline, 64 (85%) achieved cure of S-VGEI after an overall follow-up time of 57 years. Development of rifampicin resistance was not associated with a reduced cure rate (Figure S3B).

## Discussion

Our findings suggest that rifampicin treatment should be started after wound closure, owing to a 14-fold increased risk of secondary rifampicin-resistant staphylococci while having open wounds. Among patients with susceptible staphylococci at S-VGEI diagnosis, there was a trend of decreased survival among patients with secondary rifampicin-resistant isolates. However, neither overall survival nor cure were impacted by rifampicin treatment or secondary rifampicin resistance, respectively.

A comparison of our results with other studies is difficult since other prospective VGEI cohorts are scarce.[10,14] Earlier studies assessed the impact of rifampicin treatment as a part of combination antimicrobial therapy for S-VGEI.[10] However, those authors initiated rifampicin only after removal of all drains with the intention of reducing the risk of selecting rifampicin-resistant mutants. Moreover, they used a rifampicin equivalent of 1200 mg/day for a 60 kg individual and thus a higher rifampicin dosage than we did. Unfortunately, information on re-operative surgery, open wounds and/or rifampicin resistance at baseline and follow-up was not provided.

The optimal time-point for rifampicin treatment start is still a subject of debate in foreign body infection in general[3,9,11,12,19] and in S-VGEI in particular.[1,6,10] Some authors advocate early rifampicin treatment due to its rapid activity on susceptible biofilm staphylococci[20–22] emerging soon after attachment on the implant surface.[20,23] Other authors favour a delayed start of rifampicin after wound closure, removal of drains and cessation of

**Table 1.** Baseline characteristics of 143 patients with detection of overall 513 *Staphylococcus* spp. at diagnosis of vascular graft/endograft infection with or without rifampicin treatment

| Characteristic | Total ($n = 143$) | RIF treatment ($n = 82$) | No RIF treatment ($n = 61$) | *P* value |
|---|---|---|---|---|
| Male sex, *n* (%) | 117 (82) | 66 (80) | 51 (84) | 0.401 |
| Age, years, median (IQR) | 68 (60–75) | 69 (61–75) | 67 (59–77) | 0.78 |
| BMI, kg/m$^2$, median (IQR) | 26 (23–29) | 27 (23–29) | 25 (22–28) | 0.16 |
| Graft material[a], *n* (%) | | | | |
| Polytetrafluoroethylene, *n* (%) | 52 (36) | 31 (38) | 21 (34) | 0.41 |
| Polyethylenterephthalate, *n* (%) | 65 (45) | 39 (48) | 26 (43) | 0.34 |
| Biological material, *n* (%) | 35 (24) | 16 (20) | 19 (31) | 0.08 |
| Other, *n* (%) | 9 (6.3) | 5 (6.1) | 4 (6.6) | 0.58 |
| Location of graft | | | | <0.001 |
| Peripheral and groin, *n* (%) | 27 (19) | 10 (12) | 17 (28) | |
| Abdominal aorta, *n* (%) | 82 (57) | 44 (54) | 38 (62) | |
| Thoracic aorta, *n* (%) | 34 (24) | 28 (34) | 6 (10) | |
| Infect operation | | | | |
| Debridement ± NPWT, *n* (%) | 65 (45) | 38 (46) | 27 (44) | 0.85 |
| Total graft replacement, *n* (%) | 49 (34) | 26 (32) | 23 (38) | |
| Partial graft replacement, *n* (%) | 12 (9.0) | 7 (9.0) | 5 (8.2) | |
| Conservative, *n* (%) | 17 (12) | 11 (13) | 6 (9.8) | |
| Microbiology | | | | 0.002 |
| CoNS, *n* (%) | 100 (70) | 48 (59) | 52 (85) | |
| *Staphylococcus aureus*, *n* (%) | 25 (17) | 21 (26) | 4 (6.5) | |
| *S. aureus* + CoNS, *n* (%) | 18 (13) | 13 (16) | 5 (8.2) | |
| Antimicrobial therapy[b] | | | | NA |
| Monotherapy[c], *n* (%) | 47 (19) | 21 (14) | 26 (26) | |
| Other combination[d], *n* (%) | 91 (37) | 33 (23) | 58 (58) | |
| RIF combination therapy[e], *n* (%) | 82 (35) | 82 (100) | NA | |
| No treatment, *n* (%) | 34 (14) | 18 (12) | 16 (16) | |

Abbreviations: RIF, rifampicin; BMI, body mass index; CoNS, coagulase-negative staphylococci; PO, per os; IV, intravenous; NA, not applicable.; NPWT, negative pressure wound therapy.

[a]Multiple graft materials per patient possible.

[b]Multiple antimicrobial therapies per patient possible ($n = 254$; combination stated below if >5 times used). Most patients—even if they later received rifampicin or another combination therapy—started with an empirical monotherapy.

[c]Monotherapy: Amoxicillin/clavulanic acid IV ($n = 23$); Piperacillin/tazobactam IV ($n = 7$); Vancomycin IV ($n = 5$).

[d]Other combination: Piperacillin/tazobactam, aminoglycoside IV ($n = 5$); Vancomycin, aminoglycoside IV ($n = 7$); Vancomycin, ciprofloxacin IV ($n = 9$); Vancomycin, ertapenem IV ($n = 6$); Amoxicillin/clavulanic acid, ciprofloxacin PO ($n = 7$); Ciprofloxacin, clindamycin PO ($n = 11$).

[e]Rifampicin combination therapy: Flucloxacillin, aminoglycoside, rifampicin IV ($n = 8$); Flucloxacillin, rifampicin IV ($n = 9$); Vancomycin, aminoglycoside, rifampicin IV ($n = 5$); Vancomycin, ciprofloxacin, rifampicin IV ($n = 6$); Vancomycin, rifampicin IV ($n = 5$); Amoxicillin/clavulanic acid, rifampicin PO ($n = 6$); Ciprofloxacin, rifampicin PO ($n = 6$).

bacteraemia.[10,11] The theoretical basis for delayed treatment is provided by experimental biofilm studies that demonstrated a selection of rifampicin-resistant mutants of CoNS when challenged with rifampicin at or above the MIC.[24,25] By analogy implant-preserving approaches with debridement, open wound treatment and second-look surgeries may constitute a risk for rifampicin resistance due to the biofilm-associated nature of these infections.

Indeed 65% of our patients treated with NPWT and rifampicin combination therapy had any rifampicin-resistant staphylococci. Moreover, the NPWT foam may be an ideal niche for skin bacteria to form biofilm and inoculate the wound. In a retrospective study of patients with prosthetic joint infections with rifampicin-resistant staphylococci, three or more previous surgical revisions and rifampicin treatment started despite high initial bacterial load were associated with secondary rifampicin resistance[25] despite ongoing combination therapy. Similarly, we now found an association of any rifampicin resistance with rifampicin treatment during open wounds.

We could neither establish a link between indwelling drains nor between bacteraemia and the selection of rifampicin-resistant staphylococci,[11,12,19,24] possibly due to low numbers. rifampicin-resistant mutants deriving from the skin microbiota might be selected around drains or exuding wounds by antimicrobial therapy containing rifampicin.[19] Rifampicin resistance might also occur during an ongoing bacteraemia.[11] In a retrospective case–control study including 84 patients with *S. aureus* native-valve endocarditis, in 56% of cases that were bacteraemic at initiation of rifampicin therapy, rifampicin-resistant strains were isolated

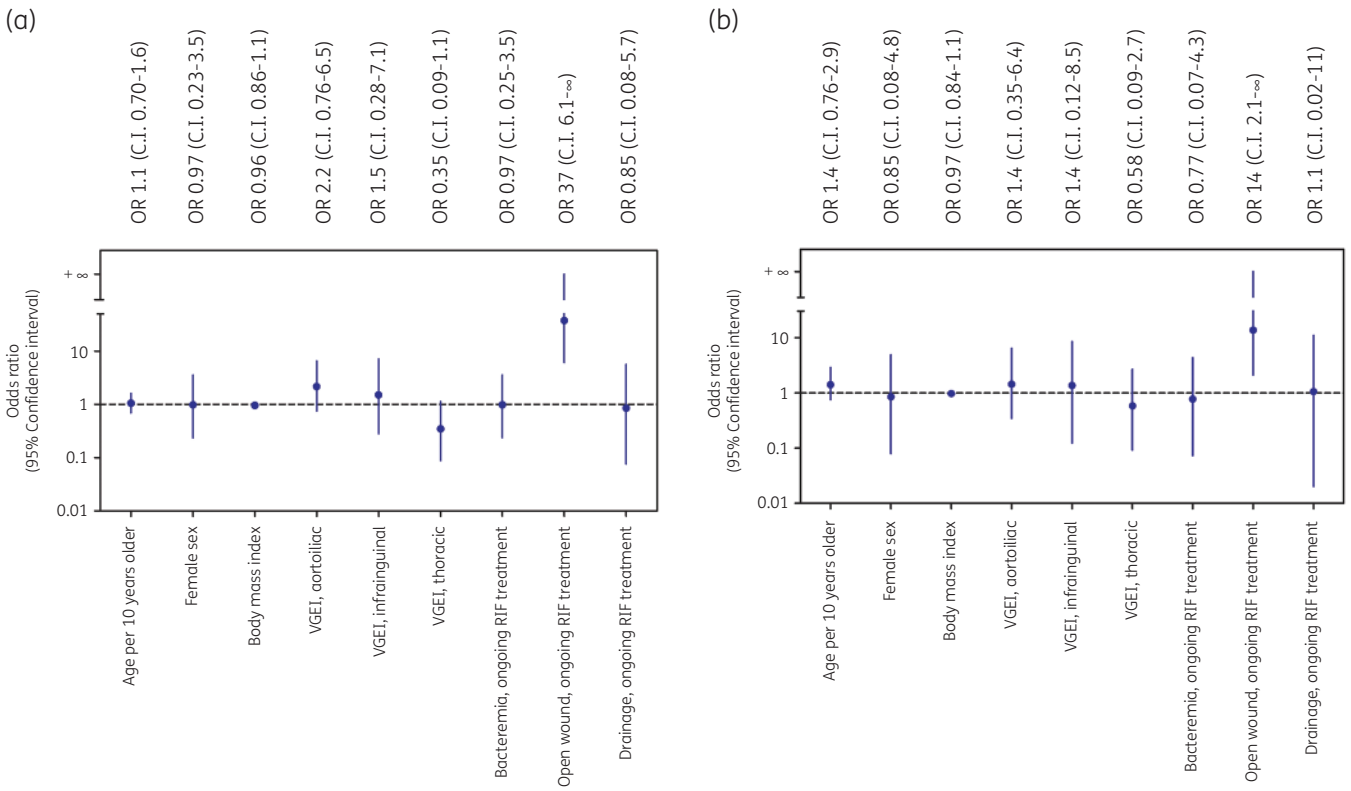


**Figure 1.** Univariable exact logistic regression to identify potential associations of rifampicin treatment with any rifampicin-resistant staphylococci (a) or secondary rifampicin resistance with susceptible staphylococci at baseline (b). VGEI, vascular graft/endograft infection, RIF, rifampicin; OR, odds ratio.

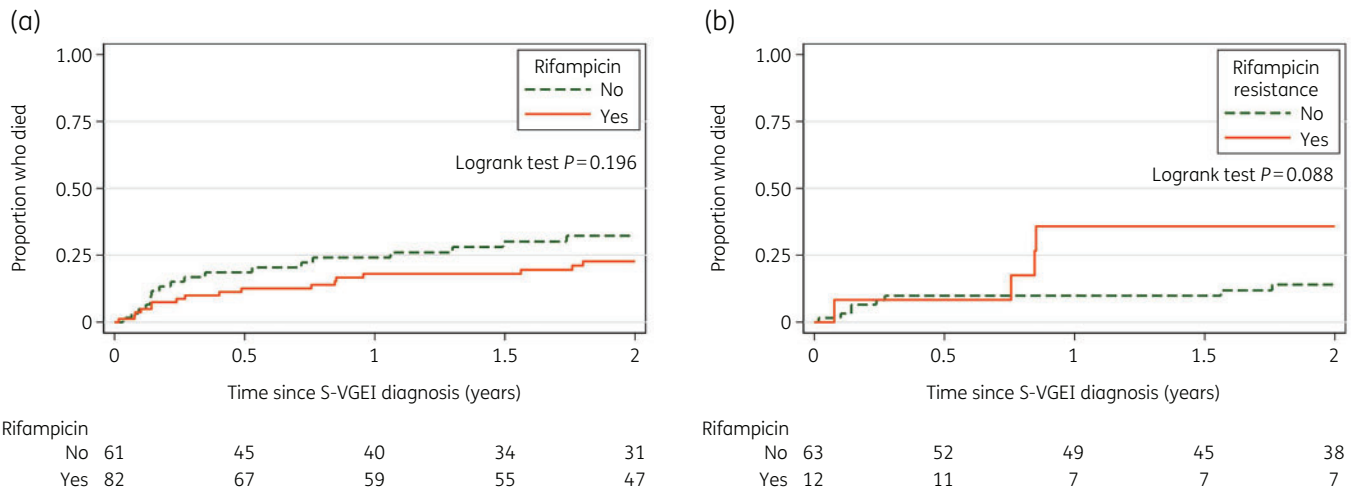


**Figure 2.** Survival analyses of 143 participants with S-VGEI stratified by rifampicin treatment (a) and 75 participants with rifampicin susceptible staphylococci at S-VGEI diagnosis and secondary rifampicin resistance (b). Note: survival time was censored at 2 years.

while there were no resistant isolates among cases who received rifampicin after clearance of bacteraemia.[12]

We used targeted drug combination therapy for all patients and despite this fact, in 12/75 (16%) patients with a rifampicin-susceptible isolate at baseline secondary rifampicin-resistant staphylococci were isolated under therapy. This contrasts with a randomized controlled study on orthopaedic device infections that showed that rifampicin-containing regimens are able to cure staphylococcal implant-related infections without any incident rifampicin-resistant isolates.[9] One of the main aims for using antibiotic combination therapy is prevention of antibiotic resistance.[26] However, evolving tolerant mutations might precede rifampicin resistance despite combination therapy[26,27] and accordingly the benefits of combination therapy in preventing resistance are

lost.[26] The evidence for use of combination antimicrobial therapy in S-VGEI and *S. aureus* bacteraemia is limited,[1,6,7] whereas guidelines on infective endocarditis recommend combination therapy[11] despite the fact that a single dose of rifampicin might be associated with rifampicin resistance, especially in MRSA endocarditis.[28]

Data from experimental VGEI suggest[20,23] that the bactericidal activity of anti-staphylococcal agents is influenced by the pharmacological characteristics of the individual agent and the graft material. In contrast, our study did not find an association of rifampicin resistance and the graft material. In contrast to other studies,[10] we did not find evidence of an impact of rifampicin treatment on survival or cure rates among patients, which is actually counter-intuitive since rifampicin is otherwise considered a biofilm-active substance especially in early S-VGEI. There was a trend of a decreased survival among patients with rifampicin-resistant staphylococci at S-VGEI diagnosis. However, time to cure was not affected among this subset of patients, although 65 patients were treated with debridement and NWPT (38 with rifampicin combination and 27 without).

### *Strengths and limitations*

To our knowledge, this study is the first clinical study that examines potential associations of rifampicin resistance with open wounds, drains or bacteraemia in a prospective S-VGEI patient cohort with long term follow-up. Due to the multidisciplinary approach, surgical procedures and infectious disease management were centrally coordinated by a vascular infection or endocarditis board at our institution.

Several limitations should be noted. This is a single centre study and our results may not be generalizable to other institutions. Further we present combined results from retrospectively collected information (chart reviews; 2002–12) and from the prospective VASGRA cohort (since 2013). Despite using a uniform case definition for VGEI,[1,14] our patient collective is heterogeneous including abdominal, peripheral and thoracic VGEI and two different patient collectives over a time span of 18 years. In these circumstances, surgical approaches might be different and hence might influence the clinical and microbiological results. Moreover, the specific question on secondary rifampicin resistance can only be analysed among patients with delayed wound closure, second-look surgeries or relapsing/ongoing bacteraemia, all factors that may introduce bias. Moreover, influenced by the ongoing controversy regarding the correct timepoint of rifampicin prescription in foreign body infections and the large number of patient years, the tendency for rifampicin prescription or the proportion of staphylococci with evolving rifampicin resistance may have changed over the years. Using test for trend analyses there was no evidence of changes in rifampicin prescription practices at our institution ($P = 0.67$). However, there was evidence of an association with a decreasing number of rifampicin-resistant staphylococci between years ($P = 0.05$). This could be connected with the fact that the percentage of patients with thoracic VGEI in VASGRA was higher than in the patient collective from 2002 to 2012, whereby the concept of delayed wound closure is less common in cardiac surgery. Polymicrobial infections occur in one-third of patients with VGEI, thus influencing the importance of microbiological isolates. Staphylococci isolated at VGEI diagnosis are surely more important than staphylococci isolated over time in follow-up samples. However, we took this into account using a stringent definition for the potential contaminant CoNS and excluded such samples from the analyses of rifampicin resistance. There are wide inter-individual variations in the exposures achieved with rifampicin standard doses and unfortunately, we did not measure the plasmacidal activity against the patient's organisms. However, among susceptible isolates the $MIC_{90}$ of rifampicin was $\leq$0.015 mg/L after both 24 and 48 h of incubation.

### *Conclusions*

Owing to the increased risk of a secondary resistance, rifampicin as part of an anti-staphylococcal antimicrobial therapy should be started after wound closure. Antibiotic combination therapy might prevent resistance, but circumstances leading to tolerance and evolving resistance under ongoing combination therapy should be elucidated in future *in vitro* and *in vivo* studies.

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## Transparency declarations

None to declare.

### *Author contributions*

This work is part of Y.K.S.L.'s master thesis. B.H. and Y.K.S.L. designed the study. B.L. analysed the data. B.H. and Y.S.L. wrote the first draft, and S.D.B., A.S.Z. and B.H. and the other authors wrote the final version of the manuscript. All investigators contributed to data collection and interpretation of the data, reviewed drafts of the manuscript, and approved the final manuscript.

## Supplementary data

Figures S1 to S3 are available as Supplementary data at *JAC* Online.

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