Author's response to reviews

Title: Endocarditis caused by methicillin-susceptible Staphylococcus aureus with reduced susceptibility to vancomycin?: First case report in Argentina.

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Endocarditis caused by methicillin-susceptible Staphylococcus aureus with reduced susceptibility to vancomycin?: First case report in Argentina.

Beatriz E Perazzi, Natalia Bello, Marta Mollerach, Carlos A Vay, María B Lasala and Angela M Famiglietti
The changes are written in red in the revised manuscript.

THE EDITOR would also like you to address the following specific points:

Question 1. Is this a case of recurrent MSSA bacteremia in a patient who failed vancomycin due to a vancomycin-heteroresistant strain S. aureus (hVISA), or a relapse after vancomycin monotherapy of endocarditis complicated with valvular abscess and spondylodiskitis with possible paravertebral infection?

Vancomycin has poor bone penetration, and so it is possible that monotherapy with this antibiotic not able is to sterilize bone. Therefore, an increased risk of recurrence exists after vancomycin treatment for S. aureus osteomyelitis, especially in cases with undrained abscesses and with short-duration parenteral antimicrobial treatment.

Answer: The persistent bacteremia could be due to different reasons:

1) The following was added in red in the discussion page 5, lines 27-29: “This persistent bacteremia could be due to the presence of a metastatic infectious focus, such as that in the left iliopsoas muscle. Since vancomycin has poor bone penetration, the initial monotherapy with this antibiotic could not have been able to sterilize bone, especially with short-duration parenteral antimicrobial treatment.”

In this manuscript is reported that the persistent bacteremia could be due to the presence of a metastatic infectious focus, as that of the left iliopsoas muscle. However, the needle puncture of the muscle did not show microbiological development?. The negative culture of the CT-guided percutaneous biopsy and aspiration of the lesion in the left iliopsoas muscle of this patient can not exclude the presence of a metastatic infectious focus, because this procedure has a very low diagnostic sensitivity, especially if the
patient is treated with antibiotics.

Answer: The following was added in red in the discussion, page 5, lines 29-32::
“Besides, the negative cultures resulting from the aspiration biopsy of the lesion in the muscle, can not rule out the presence of a metastatic infectious focus, because this procedure has very low diagnostic sensitivity, especially in patients pre-treated with antibiotics”.

2 )Answer::Another possible explanation of the persistent bacteremia :The following was added in red in the discussion, page 5, lines 32-34 to page 6, lines 1-11::” Another possible explanation of persistent bacteremia is the fact that in this case, the isolate showed vancomycin MIC ≥ 1 µg/ml, which could justify treatment failure, and infection control with the combined treatment. In this respect, it is worth mentioning that treatment of MSSA bacteremia with vancomycin is suboptimal, as has clearly been demonstrated in several studies. Its slow bactericidal activity is responsible for high probability of therapeutic failure, which increases as the MIC rises, even within the susceptibility range [3]. There are several strategies to deal with this situation. The use of high vancomycin doses in complicated infections, (25-30 mg/kg/8-12 h) to obtain trough serum concentrations of 15-20 mg/l and an AUC/MIC of >400, has shown elicit a better therapeutic response in strains with MICs ≤1 µg/ml, despite higher rates of nephrotoxicity which require serum concentration monitoring of the drug [4]. The combination of vancomycin with other antibiotics as in the present case, is another possible strategy.”

Page 8, lines 2-6: “Different lines of evidence, such as population analysis and electron microscopy, suggest that vancomycin treatment failure of the endocarditis mentioned here, could have occurred as a result of an hVISA infection. The fact that both SA isolates had clonal relationship suggests relapse and not reinfection. Besides, considering that the vancomycin doses administered in this case did not reach the recommended trough serum levels of 15-20mg/l (14.1 mg/l), it could be assumed that S.aureus subpopulations with reduced susceptibility to vancomycin might arise during therapy thus contributing to vancomycin treatment failure. However, the infection was finally resolved with vancomycin, probably because of its combination with gentamicin and rifampin”.
Question 2. Were serum levels of vancomycin appropriate (target trough levels) for this complicated infection?

**Answer:** The following was added in red in the discussion, page 8 lines 2-6: “Besides, considering that the vancomycin doses administered in this case did not reach the recommended trough serum levels of 15-20mg/l (14.1 mg/l), it could be assumed that *S.aureus* subpopulations with reduced susceptibility to vancomycin might arise during therapy thus contributing to vancomycin treatment failure. However, the infection was finally resolved with vancomycin, probably because of its combination with gentamicin and rifampin”.

Question 3. Had the patient been previously exposed to glycopeptide antibiotics?

**Answer:** The following in red was added in the Case presentation, page 3, lines 15-16: “The patient had not been previously exposed to glycopeptide antibiotics”

- Please be sure to include the patient's ethnicity both in the Abstract and Case Presentation Section.: The patient's ethnicity in the Abstract and Case Presentation Section was included in red: “Hispanic man”

Comment to the Editor: The number of references was 24, because the both reviewers solicited to include several references that were included. The new references 2, 6, 15, 16, 17, 20, 21, 24 were added in red

REVIEWER 1155601874421650

**Comments to authors:**
This is an interesting addition to the literature on the growing rate of vancomycin heteroresistant SA strains, particularly among methicillin-susceptible strains. In its present form however, there are several key omissions, as well as multiple stylistic, spelling and grammatic errors that need to be edited prior to publication. **Answer: Language corrections were made.**
Specific comments follow below.
1. The authors mention only one case of vancomycin heteroresistance on an
oxacillin-susceptible background, when in fact there are several reports of this (Fusco et al, DMID, 2009; Pillai et al, CID, 2009). These should be noted.

**Answer:** The following was added in red in the discussion, page 7 lines 27-32: “Nevertheless, Bobin-Dubreux et al [19], in France, reported a case of conjunctivitis due to MSSA in an hVISA isolate and Fusco et al [20], in USA, also reported clinical failure of vancomycin in a dialysis patient with recurrent methicillin-susceptible vancomycin-heteroresistant SA bacteremia. Besides, Pillai et al [21], reported the development of reduced vancomycin-susceptibility in a series of clinical methicillin-susceptible SA recovered from blood and bone of a patient who experienced vancomycin therapy failure”.

2. Methodology: The selection of subpopulations of vancomycin-intermediate SA by serial plating on agar with increasing subinhibitory vancomycin concentrations has been previously described (Drago, et al, Clin Micro and Infection, 2008) Moreover, this selection of subpopulations is distinct from DETECTION of resistant subpopulations, of which the most well-validated method is the PAP-AUC of the original isolates. Indeed, the criterion for HETERORESISTANCE is a PAP-AUC ratio of 0.9 or greater compared to Mu50 (Wootton, MacGowan, Walsh and Howe, JCM, 2007). While the obviously increased cell wall diameter on EM does support development of heteroresistance, one would like to see the PAP-AUC ratios as well.

**Answer:** The PAP-AUC ratios versus Mu3 of SA1, SA2 and SA3 strains were included: The following was added in red in the case presentation: page 5, lines 11-15: “SA2 and SA3 were identified as hVISA on the basis of the population analysis profiling-area under the curve (PAP-AUC) ratios, showing PAP-AUC of 1.06 and 1.26 respectively versus Mu3, whereas SA1 was identified as vancomycin-susceptible, showing PAP-AUC of 0.83 versus Mu3 [2].”

Moreover, given recent validation of the Etest GRD strip for detection of vancomycin heteroresistance (Yusof et al, JCM, 2008), this would also be interesting to note. **Answer:** The following was added in red in the discussion, page 6, lines 27-34 to page 7 lines 1-4: “Although the PAP-AUC method is considered the gold-standard method for detection of hVISA strains, it is actually too time-consuming and labour-intensive for a clinical laboratory.”
Therefore, a new Etest hGISA/GISA resistance detection (GRD) strip (E-vancomycin/teicoplanin+supplement) of recent validation in the USA was described by Yusof et al for detection of vancomycin heteroresistance [14]. The best performance for hGISA detection was found with the GRD strip on Mueller-Hinton blood with a sensitivity of 94% and a specificity of 95% at 48h, using a cutoff values of ≥8 for teicoplanin or vancomycin. The authors considered that the results for the GRD strip read after 18 to 24h of incubation, if positive for hGISA/GISA, can be reported as such, although negative results should be confirmed after of 48 h of incubation, since the sensitivity was highest at 48 h [14]. However, this method has limited availability in Argentina”.

3. Discussion: The line "It is worth mentioning that treatment of staphylococcal bacteremia with vancomycin is usually suboptimal, due to the high probability of therepeutic failure..." is misleading and should be edited to specify that treatment of Methicillin-susceptible SA bacteremia with vancomycin is suboptimal, which has clearly been demonstrated in several studies.

**Answer:** The changes are the following as the reviewer solicited: in the discussion page 6, lines 1-5: “In this respect, it is worth mentioning that treatment of MSSA bacteremia with vancomycin is suboptimal, as has clearly been demonstrated in several studies. Its slow bactericidal activity is responsible for high probability of therapeutic failure, which increases as the MIC rises, even within the susceptibility range [3]”.

b. The combination of vancomycin with rifampin has never been shown in vitro to be efficacious for the treatment of SA bacteremia. While vancomycin plus gentamicin was previously a recommendation of the Infectious Disease Society of America to hasten clearence of blood cultures, this has also recently been changed due to findings of enhanced nephrotoxicity with no real morbidity/mortality benefit (Cosgrove et al, CID, 2009). This should be noted in the text.

**Answer:** The following was added in red in the discusión, page 6 lines 14-18: “However, the combination of vancomycin with gentamicin was previously a recommendation of the Infectious Disease Society of America and the American Heart Association to hasten clearance of blood cultures, but this has also
recently been changed due to findings of enhanced nephrotoxicity with no real morbidity/mortality benefit [6]."

c. The authors conclude that "the role of daptomycin in the treatment of staphylococcal endocarditis is not clearly defined and the clinical experience and availability is limited" Indeed, the role of daptomycin in the treatment of RIGHT SIDED staphylococcal endocarditis has been well described (Fowler et al, NEJM, 2006). Moreover, the clinical experience with daptomycin in \textit{S. aureus} endocarditis is growing (Levine, JAC, 2008), and it is readily available in the US. Thus the statement should be edited to "the role of daptomycin in the treatment of left-sided staphylococcal endocarditis is not clearly defined and availability in Argentina is limited"

\textbf{Answer:} The changes are the following as the reviewer solicited: in the discussion page 8, lines 9-13: "Treatment failures of \textit{S. aureus} endocarditis, with other therapeutic alternatives such as linezolid and daptomycin have been reported [22, 23]. Although the clinical experience with daptomycin in \textit{S. aureus} endocarditis is growing [24], the role of this antibiotic in the treatment of left-sided staphylococcal endocarditis is not clearly defined and its availability in Argentina is limited."

4. Conclusion - The authors state that this work ... "warns about the need to determine the vancomycin MIC for \textit{S. aureus} as a screening method to detect the presence of hVISA, VISA and VRSA strains..." While recent lowering of the CLSI breakpoint for VISA and VRSA strains do mean that MICs are an efficient and sensitive screen for intermediate and overt vancomycin resistance, MIC testing has been shown to be notoriously insensitive in detecting hVISA isolates – the very reason why many microbiology labs in the US, including that of the New York Hospital - routinely screen all MRSA isolates for heteroresistance using the Etest GRD strip analysis (Rybak et al, JCM, 2008).

\textbf{Answer:} The changes are the following as the reviewer solicited: in the conclusion page 8, lines 20-30: “Furthermore, this work warns about the existence of these strains strains, which despite showing vancomycin MIC values of $\leq$ 2 $\mu$g/ml, considered to be susceptible by the CLSI [2], usually show vancomycin MIC values between 1 and 2 $\mu$g/ml that could be responsible for
treatment failure in severe infections if the trough serum concentrations of this antibiotic are lower than 20 µg/ml. Therefore, the correct management of severe S. aureus infections with vancomycin requires careful monitoring of the treatment by performing vancomycin MIC and its trough serum concentrations in order to adjust the treatment. Furthermore, these findings raise awareness of the need to have an adequate screening method for the detection of heteroresistant-vancomycin strains that could be adapted to clinical laboratories in Argentina.

REVIEWER 1711466550417065:
Comments to authors:
Please find below my comments on your manuscript entitled "Endocarditis caused by oxacillin susceptible Staphylococcus aureus with reduced susceptibility to vancomycin? First report in Argentina: a case report"
1) Quality of English is a serious impediment to understanding: the text should be re-addressed (including title) in order to achieve international standards of writing. Answer: Language corrections were made, including the title: “Endocarditis caused by methicillin-susceptible Staphylococcus aureus with reduced susceptibility to vancomycin?: First case report in Argentina”.
The word oxacillin was changed by methicillin in all the manuscript

2) This case presents severe methodological issues: i) Initial TEE revealed vegetations" with abscess", a finding not confirmed in the following TEE. However, cardiovascular surgery is indicated in case of endocarditis with valve abscess ( ESC & ESCMID guidelines, Eur Heart J, 2009).
Answer: The cardiovascular surgery was not made, because the abscess on the anterior leaflet of the mitral valve was not detected in the others TEE.
The following was added in red in the case presentation page 4, lines 7 “Subsequent echocardiograms were performed 15 days after start of the treatment, not revealing any changes in the vegetation size and not showing any abscess".
ii) Spondylodiskitis as a secondary focus of *S. aureus* bacteremia was treated for a shorter period of time than recommended, a fact that might influence re-emergence of bacteremia and endocarditis.

**Answer:** The following was added in red in the discussion page 5, lines 27-29: “This persistent bacteremia could be due to the presence of a metastatic infectious focus, such as that in the left iliopsoas muscle. Since vancomycin has poor bone penetration, the initial monotherapy with this antibiotic could not have been able to sterilize bone, especially with short-duration parenteral antimicrobial treatment.”

iii) In patients pre-treated with antibiotics, negative cultures from spiner aspirates cannot exclude staphylococcal infection.

**Answer:** The following was added in red in the discussion, page 5, lines 29-32: “Besides, the negative cultures resulting from the aspiration biopsy of the lesion in the muscle, can not rule out the presence of a metastatic infectious focus, because this procedure has very low diagnostic sensitivity, especially in patients pre-treated with antibiotics”.

iv) Methodology of heteroVISA identification should be more analyzed. Time kills need clarification, especially for SAS-3 strain that is not mentionned into the text, only in the figure.

**Answer:** The PAP-AUC ratios versus Mu3 of SA1, SA2 and SA3 strains were included: The following was added in red in the case presentation: page 5, lines 11-15: “In the population analysis of SA2 and SA3, a development of colonies of up to 3 and 4 µg/ml respectively was observed, with growths between 1 and 4 µg/ml, 2 to 4 more logarithms than in SA1, which developed up to 2 µg/ml. (Figure 2). SA2 and SA3 were identified as hVISA on the basis of the population analysis profiling-area under the curve /PAP-AUC) ratios, showing PAP-AUC of 1.06 and 1.26 respectively versus Mu3, whereas SA1 was identified as vancomycin-susceptible, showing PAP-AUC of 0.83 versus Mu3 [2].”

v) HeteroVISA strains sometimes present a reduced susceptibility to rifampicin. Recent data on h-VISA bacteremia and endocarditis (eg Bae et al, JID...
The following was added in red in the discussion, page 7 lines 5-21:

“The clinical impact of vancomycin treatment on these isolates is controversial. Musta et al [15] compared the vancomycin MIC by Etest and the frequency of hVISA for all MRSA blood isolates and correlated the results with the clinical outcome, detecting hVISA in 30% and 80% isolates with vancomycin MIC of 2 and 3 µg/ml respectively. The MIC of ≥ 2 µg/ml was associated with a higher mortality rate. However, the impact of vancomycin MIC and hVISA status did not affect mortality or persistent bacteremia. Bae et al [16] characterized patients with IE using a multinational collection from MRSA with and without hVISA, reporting that the patients with hVISA had a higher rate of persistent bacteremia and congestive heart failure but the mortality did not differ between hVISA and non-hVISA-infected patients. Moreover, hVISA isolates were genotypically similar to non-hVISA isolates. Also, Maor et al [17] compared patients who had hVISA bacteremia with patients who had MRSA bacteremia, reporting that hVISA bacteremia was significantly associated with prolonged bacteremia duration, greater rates of complications such as endocarditis and osteomyelitis, and emergence of rifampin resistance, compared with MRSA bacteremia. However, no significant difference in mortality existed between patients with hVISA bacteremia and those with MRSA bacteremia. Several authors have reported treatment failure with vancomycin in hVISA infections [9].”

vi) A series of vancomycin trough levels— if available— during initial treatment and treatment at re-admission would add power to the current manuscript. If the patient's vancomycin levels followed recent recommendations (IDSA executive summary, CID 2009), then the authors could suggest the presentation of an initial S. aureus sub-population. If vancomycin levels were not consistent with guidelines, then, S. aureus sub-populations might arise during therapy and trigger vancomycin treatment failure.

Answer: The following was added in red in the discussion, page 8 lines 2-6: “Besides, considering that the vancomycin doses administered in this case did not reach the recommended trough serum levels of 15-20mg/l (14.1 mg/l), it
could be assumed that *S. aureus* subpopulations with reduced susceptibility to vancomycin might arise during therapy thus contributing to vancomycin treatment failure. However, the infection was finally resolved with vancomycin, probably because of its combination with gentamicin and rifampin". 