Manuscript ID BMJ.2014.022885.R1 entitled "Trimethoprim-sulfamethoxazole vs. vancomycin for severe infections caused by methicillin-resistant Staphylococcus aureus: randomized controlled trial

Response to review comments

Please find below an answer to each comment (indented) with the main changes introduced to the manuscript highlighted in yellow.

Committee comments:

- *Please provide detailed information why only 252 of evaluated 782 patients were included (additional to the information in figure 1).*

The main determinant of the inclusion rate is who is considered eligible. This issue is not very standard across trials. Our starting point defining eligibility were all patients with clinically-significant MRSA isolates. From this set of patients the main reason for non-inclusion was treatment with study drugs >48 hrs (N=268). These were patients who were given one of the study drugs empirically or before cultures were obtained, precluding their inclusion. We could not discourage empirical treatment with vancomycin during the trial, when MRSA was suspected, as this would have been considered bad clinical practice and we considered that randomization after more than 48 hrs. of treatment would mask differences between comparators. We clarified in the manuscript the eligible population included all those with clinically-significant MRSA isolates and that empirical treatment with vancomycin was common in participating hospitals resulting in the high percentage of patients treated >48 hrs. before identification.

The second most important reason was inability to provide informed consent with no legal guardian (n=165) and refusal to participate (n=79). Both reviewers also commented on this large population of patients unable to provide consent or refusing. Of these, the first is quite unique to clinical trials of severe infections, as infection per se precludes many times the ability to provide informed consent at infection presentation, since severe sepsis may cause confusion. Many of these patients were critically ill and though conscious and aware, we judged it inappropriate and injurious to approach them for informed consent during their acute distress (recorded as inability to provide consent). In Israel the regulations are that if a patient is unable to provide informed consent, only a legally appointed guardian is allowed to provide consent in lieu of the patient (and not any relative, as acceptable in some European countries). Patients with acute infections rarely have legal guardians and thus such patients could not be included. Regarding refusal, our informed consent form specified that vacomycin is the first-line recommended antibiotic for the treatment of invasive MRSA infections while TMP-SMX is not, as per our ethics committee request. This was the reason people refusing participation stated most commonly. We added in the results section that inability to provide informed consent was mostly related to sepsis. The issue of informed consent is addressed in a sentence in discussion: “The main reason precluding patient inclusion was inability to obtain informed consent at the time of acute sepsis. In Israel, regulations allow only legal guardians to replace patients’ informed consent and with acute conditions most patients did not have an appointed legal guardian.” A lot can be said about Good Clinical Practice regulations and the balance between patients’ autonomy and the ability to conduct research. Personally, I believe that for effectiveness research the main supervision should be that of the ethics committee and patients’ informed consent procedures should be modified. However, we thought it better not to go into this discussion. We added a concise statement in the limitations paragraph of the discussion that “Patients excluded due to inability to provide informed consent had higher severity of illness scores than those included, affecting the trial’s external validity.” We actually conducted a separate analysis comparing between patients included in the RCT and those excluded due to inability to

provide informed consent showing that the latter were a sicker group of patients – we plan to publish this analysis separately.

Finally, MRSA is a common infection in haemodialysis patients in Israel and 49 patients were excluded for this reason.

- *Isn't the non-inferiority margin of 15% quite wide for a composite outcome that includes death?*

This is the currently recommended non-inferiority (NI) margin for trials of complicated skin and soft tissue infections in the FDA guidance to the industry (Justification of Non-Inferiority Margin for the Treatment of Complicated Skin and Skin Structure Infections, FDA Briefing Document for Anti-Infective Drugs Advisory Committee Meeting November 18, 2008). But our trial included significantly sicker patients and the proportion of patients reaching the outcome because of death was much larger than in cSSTI trials. In the daptomycin RCT (Fowler et al. N Engl J Med 2006) the non-inferiority margin was 20% but the outcome was largely determined by treatment modifications or other soft events. General guidance on non-inferiority margin definition is to preserve 50% of the efficacy of the "gold-standard" comparator relative to placebo/no therapy (Spellberg et al. Clin Infect Dis. 2009 Aug 1;49(3):383-91.) We don't know the effect of vancomycin vs. placebo for invasive MRSA infections, but presumably 15% would be smaller than 50% of this effect. But again, the general guidance probably does not address the outcome of mortality – for mortality one would probably want to conserve 100% of this effect.

Generally, we agree with our NI margin seems large for a composite outcome that includes mortality and is largely determined by mortality. The outcome of "all-cause" mortality includes non-infectious related deaths to a small degree and the non-inferiority margins should take this into account. Practically, a smaller NI margin would have meant an unrealistic sample size for this unfunded trial. We could not prove non-inferiority with this margin, thus the fact that it is too permissive has less of an impact on conclusions than if we were to conclude on non-inferiority. We prefer not to address this in the paper.

- *What does your paper add to Markowitz N et al. TRIMETHOPRIM-SULFAMETHOXAZOLE COMPARED WITH VANCOMYCIN FOR THE TREATMENT OFSTAPHYLOCOCCUS-AUREUS INFECTION. ANNALS OF INTERNAL MEDICINE 1992; 117:390-398*.

Markowitz's trial included intravenous drug users (IVDUs). Staphylococcus aureus infections among IVDUs are very different than in other populations, being milder infections, as they affect typically young people, and are associated with low mortality even if resulting in endocarditis (right-sided typically). Indeed, the mean patients' age in this trial was 32.5 years while in ours it was 65.8 years. All patients with MRSA infections were cured in Markowitz's trial while mortality in our trial was 25.3%. Overall, Markowitz's trial lent support to the use of TMP-SMZ for MRSA bacteremia, as in the subgroup of patients with MRSA bacteremia all patients were cured regardless of treatment assignment. Our trial suggests a different result. We deleted the reference to Markowitz's trial in the background and expanded its description in comparison with our trial in the second paragraph of the discussion.

- *The analysis could be simpler and easier to understand: You report a PP analysis as well as ITT, p-values in Table 1, a table of adverse events which is not cited (Table 3), and a multivariable logistic regression (Table 4) which is superfluous.*

To improve readability:

- We deleted p values and report only risk ratios or median differences with 95% confidence intervals. The continuous outcomes are reported as median rather than mean differences, because they had skewed distribution as expected (fever and hospitalization duration). We added the method used for the calculation of the 95% confidence intervals for the median difference.
- We reformatted Table 2 so that the ITT and PP analyses appear one below the other. We predefined these analyses in the protocol and believe that readers would like to see how many patients adhered to the trial regimens and fully fulfilled inclusion criteria and the results for them. If the editor deems it unnecessary, we can delete results for the PP population and provide in results just the number of patients in the ITT population that did not adhere to the protocol
- We corrected the omission of Table 3 in text
- We deleted the multivariable analysis for mortality. However, we would like to ask to retain the multivariable analysis for the primary outcome – treatment failure. Despite randomization there remain small differences between patient groups and conducting an adjusted analysis despite randomization provides further insight to the straightforward analysis of the RCT. It is a much stronger analysis than an adjusted analysis performed on an observational cohort since there is no selection bias.
- We tried to simplify the result's text

- *The methods don't list the variables considered in the logistic regression, and there is a list of variables on page 11 that are excluded as they are correlated with others (?).*

We added in methods that correlated variables (Spearman correlation >0.5) were not entered into the multivariable analysis. We clarified in results text on page 11: we first list the variables that were significantly associated with treatment failure on univariate analysis but were not entered into the multivariable analysis due to correlations. We then refer to Table 4 (corrected numbering) to the other variables that were included. We hope this is clearer. If the editors would like we can provide a complete univariate analysis in a supplementary file.

- *Figure 1 indicates that 25+21 patients were excluded from the ITT analyses, yet the results in Table 2 include them. Not clear what Figure 1 means.*

We meant that 25+21 were excluded when performing the per protocol analysis of patients receiving at least 7 days of the assigned treatment and not having post-randomization exclusion criteria. We clarified the text in the figure.

- *Please discuss the high dose of TMP-SMZ being used.*

We based the dosing on the dose used in Markowitz's study and a pharmacokinetic (PK) study [1]. In the PK study, a dose of 240 mg trimethoprim/ 1200 mg sulfamethoxazole Q 12 hrs. resulted in mean trough levels of 0.81/ 37.8 on day 1 and 2.64/ 78.4 on day 4 of treatment, for TMP and SMZ, respectively. The trough on day 1 was lower than the typical minimal bactericidal activity for MRSA in participating hospitals, thus the chosen dose was higher than that used in the PK study. We added this explanation to the methods section.

[1] Spicehandler J, Pollock AA, Simberkoff MS, Rahal JJ, Jr. Intravenous pharmacokinetics and in vitro bactericidal activity of trimethoprim-sulfamethoxazole. Rev Infect Dis 1982; 4: 562-5.

- *Please report primary and secondary outcomes in accordance to the trial registry.*

We tried to adhere as close as possible to the trial's protocol and registry. There were a few outcomes that were poorly defined in the trial registry. The primary outcomes are identical, only we report on failure instead of "cure or improvement" defined in the trial registry. This maintains the RRs in the same direction for all outcomes (RR>1 in favour of vancomycin). The sample size was originally calculated based on failure rates, as appears in the submitted protocol.

I detail the secondary outcomes listed in the registry and how we addressed them.

Secondary outcomes

Planned – trial registry	**Reported**
Improved or cure without antibiotic modifications [Time Frame: 7 days]	As above, failure reported instead of "improved or cure" (called treatment failure or modification)
Modification of the anti-staphylococcal treatment within 1 week of treatment onset for perceived failure of therapy [Time Frame: 7 days]	Not reported. We can add this outcome although we believe it is not informative. Since this was a trial we made efforts to continue the assigned treatment as much as possible in the first week. Thus as an outcome we do not believe it actually reflects treatment effectiveness. The numbers are actually reported in Figure 1 (as "Received <7 days treatment" – all of these discontinued the assigned regimen due to perceived failure.
Survival at 7 days post randomization without the need for modification of the anti-staphylococcal antibiotic [Time Frame: 7 days]	Not reported for the same reasons.
Bacteriological failure, defined as persistent isolation of Staphylococcus aureus with the same phenotype 7 days after or more after treatment onset [Time Frame: 7 days]	Reported in Table 2. In was inadvertently called bacteremia duration >7 days. We corrected this to "Bacteriological failure, day 7"
Need for surgical intervention or other invasive procedures [Time Frame: 30 days]	Reported under patient characteristics in Table 1. During the trial we realized that this is a process measure rather than an outcome.
Need for central catheter removal [Time Frame: 30 days]	Reported under patient characteristics in Table 1 together with foreign body removal. During the trial we realized that this is a process measure rather than an outcome.
Persistent bacteremia [Time Frame: 30 days]	Reported at 48 hours. The time frame in the trial registry was not defined (the time frame of 30 days refers to the trial's follow-up time frame during which we monitored for persistence, but there is no actual bacteremia persistence for 30 days). In the original trial protocol we defined it as positive blood cultures after 48 hours of therapy.
All-cause mortality in ICU and in-hospital	Most patients were not in the ICU thus ICU mortality was not relevant. In-hospital mortality is very similar to the 30-day mortality

	and ultimately we believe that we should have defined only 30-day mortality as a clearer measure than in-hospital mortality.
Adverse events [Time Frame: 30 days]	Reported
Durations of fever, assigned antibiotic treatment, mechanical ventilation, ICU and hospital stay [Time Frame: 30 days]	We reported on duration of hospital stay. We added the duration of fever – we did not list it originally because the data came out not very interesting as most patients had fever when starting treatment and defervesced immediately. The other durations became irrelevant because our trial population was mostly outside the ICU and not mechanically ventilated due to the informed consent procedures specified above.
Resistance development [Time Frame: 30 days]	Reported

Reviewers' comments:

Reviewer: 1

Comments:
This is an open-label RCT designed to evaluate whether TMP-SMZ is noninferior to vancomycin for severe MRSA infections. Overall, this study is well-done and is a valuable contribution to the literature; there is a clear unmet need for high quality data in treatment of serious MRSA infections.
** Originality - does the work add enough to what is already in the published literature? If so, what does it add? If not, please cite relevant references.*
There is a need for additional data on therapeutic options for MRSA infections, especially bacteremia. There is only one prior RCT comparing TMP/SMZ to vancomycin (Markowitz et al, cited in paper). These same authors have published a small retrospective matched study to examine the role of TMP/SMX in MRSA bacteremia (Goldberg et al, J Antimicrob Chemother 2010), the promising results of which provide justification for this RCT.
** Importance of work to general readers - does this work matter to clinicians, patients, teachers, or policymakers? Is a general journal the right place for it?*
Yes on both counts. S aureus bacteremia is common and knowledge of its treatment should be of interest to the general audience
** Scientific reliability - Research Question - clearly defined and appropriately answered?*
Yes, the question of noninferiority of TMP-SMZ to vancomycin for invasive infections is clearly defined.
Overall design of study - adequate ?
Yes. The number of bacteremia patients is small but this is a notoriously difficult population to enroll, and their sample size of N=91 MRSA bacteremia is the same as the sole high-quality RCT previously *reported (Fowler et al, dapto vs standard therapy, MRSA bacteremia N=89)*
Participants studied - adequately described and their conditions defined? Yes
Methods - adequately described? Complies with relevant reporting standard - Eg CONSORT for randomised trials ? Ethical ?
Methods adequately described. Would like clarity around whether all bacteremic patients had documentation of clearance of bacteremia (see below) and information on concomitant antibiotic therapy. I see no ethical concerns.

Addressed below. Yes, all bacteremic patients had documentation of clearance of bacteremia. Information on concomitant antibiotics added.

Results - answer the research question? Credible? Well presented?
The research question is answered – TMP-SMZ did not meet noninferiority criteria.
Interpretation and conclusions - warranted by and sufficiently derived from/focused on the data? Message clear? Yes. Would be more circumspect length of stay data as TMP-SMZ did not have a clear advantage in LOS in this study or in the prior studies cited.

We re-expressed results for LOS as median difference and modified text to say that there was no significant difference between treatment arms.

References - up to date and relevant? Any glaring omissions?
- *Could add their own prior observational study as noted above (Goldberg et al, J Antimicrob Chemother 2010)*
- *Recent JAMA review examining treatment options, including TMP/SMZ, for MRSA bacteremia (Holland et al, JAMA 2014)*

Added missing references to introduction.

Abstract/summary/key messages/What this paper adds - reflect accurately what the paper says? yes

Other specific comments:
-the authors are to be commended for the high proportion of screened patients that were enrolled. It does seem likely that sicker patients were more likely to be excluded, since N=165 were excluded as they were unable to provide consent. Could comment on this

As above. Yes, patients unable to provide informed consent were sicker than those that could be included. We added in results inability to provide informed consent was mostly related to sepsis and in the limitations paragraph of the discussion we add that "Patients excluded due to inability to provide informed consent had higher severity of illness scores than those included, affecting the trial's external validity."

Page 4, paragraph 3 – would reword the comment on resistance to vancomycin. Resistance to vanc remains very uncommon; there is healthy debate about whether vancomycin is the optimal agent for infections due to MRSA with vancomycin MIC at the higher end of the susceptible range (e.g. MIC=2), however resistance (MIC>=4) remains rare. The role of TMP/SMZ for vanc MIC=2 has been specifically explored in observational fashion by Campbell et al, Annals Pharmacother 2012

The comment on resistance to vancomycin reworded in the background. Resistance to vancomycin deleted, as it is indeed uncommon, and we addressed the issue of vancomycin at the higher end of the susceptible range. Campbell's study on the effectiveness of TMP-SMZ for vancomycin MIC=2 added to introduction.

Page 5, paragraph 1 – several inaccuracies here - I don't think that sample size is correct. In Markowitz, there were 38 patients with MRSA bacteremia, of 65 total patients with bacteremia. Also, hospital stay was not significantly different in those with MRSA infection, and the duration results were compared for all patients with MRSA infection, not just bacteremia.

In *Markowitz*'s trial 228 patients (intravenous drug users) with suspected Staphylococcal infections were randomized and 101 with proven staphylococcal infections (56 MSSA and 47 MRSA) were evaluated for clinical efficacy. There were 65 patients with bacteremia, but there is a confusion probably regarding the number of patients with MRSA bacteremia, as in the footnote to Table 4 the number of patients with MRSA bacteremia treated with TMP-SMZ is larger than the number of total patients with MRSA infections treated with TMP-SMZ. Therefore, we revised the description of this study quoting the total number of patients MRSA infections rather than the number with bacteremia. Indeed, hospital stay refers to all patients with MRSA infections. We deleted this result as both reviewers commented on it, the result was not statistically significant and irrelevant to current hospitalization practice. We moved the description of the study to the discussion only.

Methods: I am curious why patients with CNS infections were excluded – TMP/SMZ is likely inferior for MSSA meningitis but is a reasonable option for therapy in MRSA infections

Although TMP-SMZ has good CNS penetration, the data available for the effectiveness of TMP-SMZ specifically for MRSA CNS infections - meningitis or brain abscess - are very limited. I believe it is limited to case reports, mostly of salvage therapy. We considered that it might not appropriate to include these patients in a RCT and would introduce unnecessary heterogeneity to the trial population. In any case the number of patients with MRSA CNS infections is very small in the trial settings and actually only 3 patients were detected throughout the study period with MRSA meningitis and excluded for this reason.

Would acknowledge that vancomycin dosing was less aggressive than current guidelines recommend for severe infections (target trough 15-20, higher loading doses for seriously ill patients – Rybak et al 2009), and that the vancomycin dosing regimen in this trial may thus have underestimated the efficacy of vancomycin.

Added to the limitations paragraph: "the dosing of vancomycin and trough levels achieved (≥ 15 µg/mL only in approximately half of patients) were lower than currently recommended (20), potentially resulting in an underestimation of the efficacy of vancomycin and the difference between arms." Dosing in the study was planned according to available guidance at the start of the trial. In recent years guidelines indeed recommend higher dosing and higher trough concentrations.

Concomitant antibiotics were allowed – what proportion of patients in each group received other antibiotics that may have been potentially effective?

Antibiotics covering the MRSA isolate other than the allocated antibiotic were given to 14/135 (10.4%) in the TPM-SMZ arm and 8/117 (6.8%) of patients in the vancomycin arm (p=0.32). These included rifampin (11), clindamycin (5), chloramphenicol (2), gentamicin/ amikacin (2) and minocycline (1) with very small numbers more or less equally distributed between groups (one patient received both gentamicin and rifampin). None were started at onset of allocated treatment – they were added to the study drug sometimes as a response to treatment failure and it is difficult to adjudicate treatments to failure or non-failure modifications. We added to results the total number of potentially effective antibiotics added per study group and revised in the discussion the limitation regarding additional antibiotics.

Please clarify – you state "blood cultures were repeated daily for patients with persistent fever or other signs [of] infection" – were there patients with bacteremia who did not have clearance of bacteremia documented (ie no negative cultures)? If so, how was duration of bacteremia calculated for these patients?

We corrected this sentence which was not precise: “Blood cultures were repeated on day 2 for all patients with bacteremia and daily thereafter for patients with persistent fever, persistent bacteremia or other signs of infection.”

What proportion of patients in the vancomycin arm had trough levels >=15?

Added to results: 65/97 (55.6%) patients treated with vancomycin had trough levels >=15.

Page 12, paragraph 1, line 25 – typo “TMP-TMZ”

Corrected.

Page 13 – paragraph 2 – in reference to Markowitz study – duration of hospital stay was not different overall between TMP/SMZ and vancomycin (24.6 vs 24.7 days) and even in the MRSA subgroup was not statistically different – would not use that study as evidence that TMP-SMZ use is associated with shorter hospital stay

As above, revised the description of this study and omitted the statement regarding hospital stay.

Page 13, paragraph 3 – would update the list of treatment options to include the newer agents approved for ABSSSI – tedizolid, dalbavancin, oritavancin

Added the newer treatment options. To more concisely address the evidence for the efficacy of all newer antibiotics, we deleted the details on trials that assessed only skin and soft tissue infections and we addressed individually only RCTs on other types of infections, more similar to our trial.

Page 14, paragraph 1 – in the Fowler daptomycin study, there were 246 patients overall, 235 in mITT population. The comparison was between daptomycin and standard therapy (not just dapto vs vanc), so would be more accurate to say either “daptomycin was non-inferior to standard therapy for SAB” or “among the subgroup with MRSA bacteremia, daptomycin was noninferior to vancomycin”

Revised as suggested.

Table 1 – do you know what proportion of patients with bacteremia had complicated vs uncomplicated bacteremia?

Data were not collected in a way allowing is to separate complicated vs. uncomplicated bacteremia with current definitions.

Table 2, footnote 2 – not immediately clear what “C” and “V” refer to here… I did eventually figure out “C” is for “cotrimoxazole”, but this is not clear as presented

Corrected.

Table 2, footnote 5 – would not use the word “cotrimoxazole” as the term TMP-SMZ is otherwise used throughout the paper. Also, doubt that the acronym CRE will be familiar to the general reader – would spell out. And would consider doing the same for VISA and VRE. And again, “3C “ and “5V” are confusing terms

Corrected.

Reviewer: 2

Comments:

MRSA has been declining in many countries but remains important. Cotrimoxazole is used for more specialist indications than formerly in view of adverse effects. This study includes a reasonable number of MRSA infections given the difficulty in recruitment. While risk ratio did not indicate a difference in efficacy of the two regimens the logistic regression showed co-trimoxazole to be associated with treatment failure. This is a useful outcome though not entirely surprising. Vancomycin resistance in MRSA is rare despite the assertion in the introduction.

Criteria for healthcare acquired infections were adapted from surveillance definitions but not defined.

Treatment was given for 7 days even though most recommend a 14 day course of staphylococcal bacteremia (Lancet Infectious Diseases 2011; 11: 208–22).

The minimum treatment duration required per protocol was 7 days, but the actual duration depended on the clinical indication. We added the actual treatment duration with study drugs to the first paragraph of results (medians of 17 vs. 14 days for TMP-SMZ vs. vancomycin.

Please confirm the CONSORT criteria applied?

We followed the CONSORT recommendations for reporting. The CONSORT checklist was supplied with the manuscript.

In Table 1 p values are not useful, the one possible difference was given in text.

We agree. However, without p-values readers might ask themselves whether some of the differences are significant or not. We therefore prefer leaving the p-values but will delete them if the editor recommends so.

Table 3 explain RIFLE.

Added in footnotes an explanation.

Only 252 of 782 patients were included so that more information is needed on the excluded group to provide reassurance of no bias. The rules regarding informed consent were beyond the investigators' control but a large proportion refused consent – were these patients the more ill?

Yes, excluded patients were sicker than those included and the main reason for this was the group of patients who could not sign informed consent and did not have a legal guardian at the time of infection onset. We clarified this in the manuscript in the first paragraph of results and in the discussion.

Where differences are not statistically significant it is preferable not to say there was a difference. This applies where the difference was thought 'clinically significant'. This can be misleading as no safe inference can be drawn without statistical significance. Therefore that statement needs to be removed from the discussion and conclusion. The most that can be said is the results show a trend to a difference. The actual finding of not establishing non-inferiority and an Odds Ratio in favour of treatment failure are sufficient to suggest cotrimoxazole should not be used for severe MRSA infections.

We reworded the text addressing the non-significant results. We only kept the statement regarding the difference in mortality rates among patients with bacteremia saying that mortality was higher with TMP-SMZ, but noted that the difference was not statistically significant.