Author's response to reviews

Title: Community-onset bloodstream infection with multidrug-resistant organisms: a matched case-control study

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Author's response to reviews: see over
14\textsuperscript{th} January 2014

To,

Dr Vangelis G Alexiou  
BMC Infectious Diseases  
BioMed Central  
236 Gray’s Inn Road  
London WC1X 8HB  
United Kingdom

Dear Dr Alexiou,

Re: Response to reviewers’ comments for MS: 1409457064110899 - Community-onset bloodstream infection with multidrug-resistant organisms: a matched case-control study

Thank you for the valuable comments related to our manuscript. Our point-by-point responses to the comments are attached.

We look forward to hearing from you.

Sincerely,

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Editors Comments

A. How many variables were included in the multivariate analyses? The threshold of p<0.1 in the univariate analysis for selection of variables to be included in the multivariate analysis may be too low for this sample size. As a result, it does not seem that the rule of thumb has been followed in the stepwise logistic regression model (a minimum of 10 binary events per candidate variable inserted in the multivariable model). This may significantly affect the validity of the multivariate analysis. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR (1996) A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 49(12):1373 - 1379.

Author response: To improve clarity with regard to the multivariable models, we have now stated the covariates that were eligible to be included in the models (after excluding significant correlated variables) as a footnote to the tables. We also performed a sensitivity analysis by performing both a backward and forward selection analysis and the same independent variables were obtained. To allay any fears of overfitting, we have used the forward selection process (pg 10) and have performed a goodness of fit assessment using the Akaike information criterion (AIC) during the building of all models to ensure that over-fitting was not an issue. We also performed the Hosmer-Lemeshow goodness of fit test, which supported that overfitting was not an issue. We feel there is no compromise of the validity of our multivariate models.

Furthermore, the authors have not performed a collinearity analysis of the variables inserted in the multivariate analysis. Certain variable may be redundant and highly correlated with other variables that are already in the model. Reading through the variables, I can find some that are logically and clinically connected and maybe statistically correlated, as well. This needs to be computed as it affects the validity of the results.

Author response: As written under the statistical methods, we did assess for collinearity of all variables inserted into the multivariable model. We used a pairwise correlation matrix, and excluded correlated variables (pairwise correlation coefficient > 0.7), with only one of the covariates being selected for inclusion into the model on the basis of the strength of association. As such, severe sepsis or septic shock was excluded because the pairwise correlation coefficient was > 0.7 between this variable and with Pitt bacteraemia score, which includes hypotension as part of the score (the parameters making up the Pitt bacteraemia score have now been included in the methods to improve clarity, pg 8-9). We also used collinearity diagnostics (variance inflation factor and tolerance) during the model building process. Again, to optimise validity we have been thorough in our model building process to prevent collinearity.

Small changes in the selection criteria of variables inserted in the multivariate analysis have a huge impact on the final results. The authors may have to considering downgrading the statements and conclusions that are based on the multivariate analysis, give more importance to the presentation and discussion of the univariate analysis and the descriptive statistics of this cohort, and discuss the limitations and potential error that is introduced by the statistical methods. For me, there is no
Author response: We strongly believe that we don’t need to change any of our conclusions based on our multivariable models. We hope the responses to the above two queries have resolved your concerns. We have used standard and logical steps to model building and there should be no question over the validity of the multivariable results. Both forward and backward stepwise selection gave the same results. We will respond to Dr. Pontikis’s 8th comment below but I would like to highlight that ‘inappropriate empirical therapy’ is not a well established risk factor for death for infection caused by gram-negative bacteria.

It is best to provide a statement from the authors' group statistician about the goodness of fit of the statistical analysis.

Author response: See responses above, which come from our group’s biostatistician.

B. Clinical definition of infection and differentiation from colonisation is difficult, especially in retrospective studies. How did the authors define clinical infection? Please discuss limitations.

Author response: It is this exact reason why we chose to only study bloodstream infection. It is not difficult to differentiate infection from colonisation when you are looking at a sterile site. Furthermore, we excluded the single most important blood culture contaminant; coagulase negative staphylococci. Therefore, we feel that this is not a justifiable limitation of this study.

C. How did the authors analyse cases of multimicrobial infections?

Author response: This has been clarified in the methods (pg 8) and results section under ‘Risk factors for COBSI due to MDR organisms’ (pg 12). Cases of multimicrobial infection were excluded in our analysis (i.e. seven out of 14 excluded cases were multimicrobial infections).

D. Is the mortality analysis an all-causes mortality analysis? How did MDR COSBI and COSBI affect survival of patients?

Author response: Yes, the mortality analysis is an all-causes mortality analysis. As written in the results (pg 13), “The overall 30-day mortality observed in both groups was 13% (46 / 360), with 16% (n = 28) in those infected with MDR organisms as opposed to 10% (n = 18) in non-MDR infections (P = 0.127)”.
Reviewer 1 Comments: Pieter Depuydt

Major compulsory revisions:
None

Minor essentially revisions:
The authors should clarify a few issues.

I suppose that in the matched cohort, MRSA cases were matched with MSSA cases; or is MDR MRSA (as defined in the methods) matched with 'susceptible' MRSA?

Author response: Yes, MRSA cases were matched with MSSA cases with the closest date of ED admission.

How were non-fermenters, such as Acinetobacter, with high intrinsic resistant matched with their susceptible counterparts?

Author response: Our definition of MDR for non-fermenters specifically took into account the intrinsic resistance. For non-fermenters, MDR was defined as non-susceptible to at least 3 classes of clinically important antimicrobials that include (i) antipseudomonal penicillins, (ii) antipseudomonal cephalosporins, (iii) carbapenems, (iv) aminoglycosides, and (v) quinolones. Out of the 12 included Acinetobacters, three were considered MDR (cases) and the remainder were non-MDR (ie not resistant to at least 3 classes) and were available to be used as controls. For clarity, the word susceptible has been changed to non-MDR (pg 8, first paragraph of Risk factors and outcomes associated with MDR COBSI)

The authors should more explicitly mention the variables entered in the multivariate analysis (e.g. what exactly is meant by 'biologically plausible'). Have multiple models been tested, and what were the predictors entered in these models?

Author response: As discussed above, the covariates included in each model are now described as a footnote to the relevant tables. We have deleted ‘biologically plausible’ given that these variables were eligible for inclusion based on their P value on univariate analysis.

While the discussion gives a nice overview of the available literature published on the matter, I feel that it should be condensed a little more.

Author response: The discussion has now been shortened.
Reviewer 2 Comments: Konstantinos Pontikis

Major compulsory revisions

Data collection and definitions- i) prevalence and temporal trends of MDR organisms – 1st paragraph

1. The authors state that ‘For the majority of the study period susceptibility breakpoints were based on the BSAC guidelines. These were changed to the EUCAST guidelines from July of 2010. We observed no change in resistance patterns at the time of the change in breakpoints definitions’.

What is meant by ‘no change in resistance patterns’? Because confusion may be derived by the use of susceptibility breakpoints, MIC90 / MIC50 reporting is preferable.

Author response: ‘No change in resistance patterns’ refers to no difference in the proportion of resistant isolates as a consequence of the change. This proportion did not change after the introduction of EUCAST breakpoints, which only changed slightly for Enterobacteriaceae and Pseudomonas. This reflects the fact that not many isolates had MICs around the susceptible breakpoint. The sentence on pg 7 in the methods has been modified to improve clarity. An automated system (Vitek®) was used for susceptibility testing and therefore exact MICs were not available.

Data collection and definitions – ii) risk factors associated with MDR COBSI – 2nd paragraph

2. The authors state that ‘Sources of BSI were divided into low risk sources including urinary, skin and soft-tissue, and catheter-related, and high risk sources including respiratory, intraabdominal, bone and joint, cardiovascular or unidentified’.

What does high and low risk imply? Why does bone and joint infection represent higher risk? Which are possible cardiovascular sources of BSI? (endocarditis, endarteritis, something else?)

Author response: High and low risk sources of BSI imply the severity of the BSI and reflect the risk for mortality. A previous study has categorised the risk for different sources of BSI; for instance, low risk (associated mortality, ≤30%), included the urinary tract, intravenous catheter, and soft-tissue sources and high risk (associated mortality, >30%) included the lung, abdominal, and unknown sources (reference - Blot S, Vandewoude K, De Bacquer D, Colardyn F: Nosocomial bacteremia caused by antibiotic-resistant Gram-negative bacteria in critically ill patients: clinical outcome and length of hospitalization. Clin Infect Dis 2002, 34:1600-1606). This reference has been added (pg 8, second paragraph of Risk factors and outcomes associated with MDR COBSI). Bone and joint infection was categorised as high risk because it is often associated with a more complicated and longer course. Furthermore, bone and joint infection in the setting of BSI is defined as a complicated bacteraemia. Cardiovascular sources are defined as valve or any other endovascular source.
Results, Temporal trend of MDR organisms causing COBSI, last paragraph
3. The authors state: ‘About 10% of Streptococcus pneumoniae blood culture isolates were non-susceptible to penicillin.’
Regarding S. pneumoniae, the description of a bacterial population (such as the 104 strains of pneumococcus in this study) by means of resistance thresholds is a source of confusion (for example EUCAST proposes different thresholds in different clinical settings: #0.064 for CNS infection, and #0.5 up to #2 µg/ml for pneumonia). The use of MIC50 / MIC90 would bypass this interpretation obstacle.

Author response: Given that all of our cultures were from blood and none from CSF, the susceptibility interpretation is based on non-CNS breakpoints. This has been clarified in the methods section pg 7.

Discussion, 1st paragraph
4. The authors state: ‘…. predominately affecting younger patients with a urinary source for their bloodstream infection,…’
The authors present not enough data to support this statement. On multivariate analysis, increasing age had a protective role (by a factor of 3% per year), but among factors examined, multi-drug resistance was predominately driven by previous contact with the healthcare system. Furthermore, some data need to be presented to prove the primarily urinary source in younger patients with MDR COBSI.

Author response: This statement has been removed (pg 14).

Discussion, 1st paragraph
5. The authors state that the Friedman criteria were less predictive for MDR GNB than they were for MRSA.
The odds ratio regarding MRSA BSI was arithmetically superior the OR for MDR GNB, but the authors need to state whether this difference was statistically significant.

Author response: A significantly higher proportion of COBSI cases with MRSA (63/67, 94%) compared to cases with MDR GNB (81/109, 74%) fulfilled at least one Friedman et al criteria \((P = 0.001)\). This has been added to the results section, top paragraph on pg 13.

Discussion, 5th paragraph and Table 2
6. The authors suggest that the published risk factors may be too general for BSI and may lead to overuse of broad-spectrum antibiotics.
The authors need to acknowledge that one should rather prefer a sensitive predictor of MDR BSI in the ED setting, rather than a specific one. The cost of over-use of broad spectrum antimicrobials is rather minimal in the case of BSI, provided that a de-escalation strategy is adopted. Furthermore, disappointingly, no innovative risk factors for MDR COBSI were identified (or reported) that would increase the sensitivity of the Friedman et al criteria. (Factors that would identify MDR in the remaining 17.8% of case patients). Instead, the authors concluded to a list of well-known and epidemiologically closely inter-related risk factors for MDR infection.
Table 3
7. History of diabetes and surgery in the prior 30 days are reported as independent predictors of MDR GNB, while the p-value is >0.05.

Author response: These variables were initially included in the table because the model fit appeared best with them in despite them being non-significant. Given that the results are the same whether the non-significant variables are included in the final model or not, we have gone for a more parsimonious model of not including them. The final model includes the same four independent, significant variables.

Table 4
8. Unexpectedly, several well-established risk factors do not predict death. Apart from inactive empiric therapy (which is commented in the text), severe sepsis/septic shock and ICU admission were not predictors of a fatal outcome. How would the authors comment on that? Is it possible that the control group was not representative enough, and 1:2 or 3 case-control study would be more appropriate?

Author response: As mentioned above, inactive empiric therapy is not a well-established risk factor for death for infections due to gram-negative bacteria. There have been mixed results in the literature. Severe sepsis/septic shock was not an independent predictor because this was covered in the Pitt bacteraemia score (which was shown to be an independent predictor), and this is a scoring system that assigns points to clinical observations such as abnormal body temperature, blood pressure, receipt of mechanical ventilation, altered mental status and cardiac arrest. The description of Pitt bacteraemia score has been added on pg 8-9. ICU admission was not shown to be a significant variable in the univariate analysis because there was reverse confounding with age. Even when it was included in the model with age, it still remained non-significant and this was most likely due to other variables capturing the clinical parameters associated with ICU admission, more specifically those in the Pitt bacteraemia score.

Minor essential revisions:
Last paragraph in Methods, ii) risk factors and outcomes associated with MDR COBSI.
The reference [1] should be followed by the ‘comma’.

Author response: This has been changed.

Results, Risk factors for COBSI due to MDR organisms, 1st paragraph
The sum of MDR GNB and MRSA does not equal 180.

Author response: The sentence is not saying that the sum of MDR GNB and MRSA is equal to 180. The first sentence of the paragraph explains that there were 194 patients. It then describes that 14 were excluded, which equals 180. It then explains that of the 180, 109 cases of MDR GNB and 67 MRSA were included in the subgroup analysis, excluding 4 cases of VRE.
Discretionary revisions

Title
1. The title is a little misleading since the vast majority of MDR infections were healthcare associated (community-onset kind of points to community-acquired)

Author response: In actual fact, community-onset is the most correct term. These bloodstream infections started in the community. It doesn’t mean community-acquired. Community-onset infections can be further categorised as healthcare-associated infection and community-acquired infection.

Methods/Data collection and definitions
2. The authors state that ‘Repeat cultures from an individual patient growing the same organism within 14 days, cultures reported as normal flora or probable contaminants (eg. coagulase-negative staphylococci from a single blood culture bottle)…. were excluded.

Some clarifications are needed. How can an organism be identified in the previous 14 days, since the study population presents to the ED from the community? This exclusion criterion could also be a source of bias, since in patients with healthcare acquired bloodstream infections there is a greater probability of positive blood cultures in the 14-day period prior to their ED presentation. In other words, certain patients with healthcare acquisition and MDR etiology would thus be excluded.

Author response: This exclusion is in place to prevent the inclusion of relapses from the same infection episode. With regard to reviewer’s comment, How can an organism be identified in the previous 14 days, since the study population presents to the ED from the community? There are several scenarios where this could happen, 1) A patient can present to ED, be admitted for a short period and then go home on antibiotic therapy, or 2) A patient may present to ED and then be discharged before knowing they have a positive blood culture result and then represent days later. The exclusion criterion was also applied to isolation of the same organism in blood cultures from patients being transferred from other hospitals (with >48 hours of hospital stay) to exclude cases of hospital-acquired MDR organisms.

2. It would be more acceptable if MDR was defined according to some official document or consensus report, than previous studies in the field (references 10 and 11)

Author response: The joint initiative of the European Centre for Disease Prevention and Control (ECDC) and the Centres for Disease Control and Prevention (CDC) have recently published some definitions for MDR [Magiorakos AP, Srinivasan A, Carey RB et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18:268-281]. For the purposes of our study and taking into account the range of organisms we assessed (both gram-negative and gram-positive), the previously published definitions were more suitable and were very much in-line with what is defined in the CDC document.

3. The authors state that ‘Multi-resistant MRSA was defined as MRSA resistant
to three or more of the following antibiotics…..’
The authors need to state whether this was an arbitrary definition or derives from some reference.

Author response: This definition follows the definition applied in surveillance of S. aureus bacteraemia by Australian Group on Antimicrobial Resistance. This has been cited previously as reference 12 in the subsequent sentence. Reference has been added following the above sentence on page 7.

4. The authors state ‘The EMRSA-15-like strain was defined as…..’
Some clarification needs to be given on EMRSA-15-like strain. The general public might not be familiar with the term.

Author response: The EMRSA-15-like strain refers to another MRSA phenotype that resembles healthcare-associated United Kingdom type MRSA, with intermediate susceptibility between mMRSA and nmMRSA. This phenotype has been found to be prevalent especially in Australian nursing homes.

Results, Temporal trend of MDR organisms causing COBSI, 2nd paragraph
5. The authors state: ‘In contrast the incidence of MDR Klebsiella spp (n=11) and other Enterobacteriaceae (n=20) did not show a significant increase over time.’
It seems as if the statistical power of the study is too small to reach any conclusions about Klebsiella and Enterobacteriaceae resistance rates over time.

Author response: The trend analysis was performed using the chi-squared test for trend or linear regression (if the count frequency is small). A linear regression will regress the rate of MDR (dependent variable) vs. the year (explanatory variable), showing if there is any change in MDR rate over time (either increasing or decreasing). However, number of Klebsiella and Enterobacteriaceae isolates was small and could have had insufficient power to show a significant increase. This statement has been added on pg 11.

Discussion, in general
6. The discussion is a little long in relation to the results presented.

Author response: The discussion has now been shortened.

The comments and suggestions of the reviewers were very helpful in the revision of the manuscript. We think the manuscript has been strengthened by the suggested changes.

Thank you once again for your time and effort,

Sincerely,

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