PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Azithromycin and Survival In Streptrococcus pneumoniae Pneumonia: A Retrospective Study
AUTHORS	Shorr, Andrew; Zilberberg, Marya; Kan, Jason; Hoffman, Justin; Micek, Scott; Kollef, Marin

VERSION 1 - REVIEW

REVIEWER	Pontus Naucler MD PhD Dept of Microbiology, Tumor and Cell Biology Karolinska Institutet and Dept of Infectious Diseases, Karolinska University Hospital, Sweden I have no conflict of interest.
REVIEW RETURNED	05-Apr-2013

GENERAL COMMENTS	**General Comments** **Materials and Methods** Even though the authors perform adjustment for potential confounders I am still concerned by the risk of confounding by indication. It would strengthen the conclusions if the authors could in addition to current analyses perform adjustment for being treated with azithromycin, such as propensity score analyses. I believe the authors should carefully consider what variables to include in the multivariate model since this is not prognostic modeling but specifically investigate the effect of macrolide on mortality. Hence it is questionably if one should adjust for mechanical ventilation or ICU admission in analyses that investigate the effect of macrolide treatment (likely to have been given before MV or ICU admission) on mortality. I would suggest that that the authors use CURB-65 score in the model to adjust for severity of disease instead. The authors only include information and adjust for two co-

	morbidities, renal failure and immunosuppression. Several other factors have been associated with poor outcome in pneumococcal disease such as severe liver disease, heart disease and pulmonary disease. The results of the analyses would be strengthened if other co-morbidities would be taken into account as well. The authors should perform analyses for the effect of other antibiotics as well to show that the mortality effect is specific to azithromycin. E.g. I believe patients treated with moxifloxacin seldom received it in combination with azithromycin while patients with B-lactam antibiotics often received it in combination with azithromycin. Hence, from the current analyses it is not possible to deduce if the reduced mortality is an effect of azithromycin or B-lactam antibiotics instead of moxifloxacin treatment. **Results** The prevalence of S. pneumoniae in the pneumonia cohort will be dependent on how many patients were tested with sputum, blood culture, urine antigen etc. Please provide data on how many patients had full basic testing, i.e. blood culture, respiratory sample and urinary antigen testing. The authors state that “inappropriate therapy occurred not because of the use of an in vitro inactive agent but because a delay in the initiation of antibiotics”. Can the authors please provide data on the frequencies of antibiotic resistance to different agents. To make it easier for the reader to understand the data I would suggest that the authors divide Table 1 on page 18 into two tables, one with baseline characteristic and one with clinical management. CURB-65 score should be presented as both median and as frequencies in each category. In the table please present frequencies of patients treated with different antibiotics. Please provide exact numbers in tables, not only percentages. In Table 2 present unadjusted as well as adjusted odds ratios as

	suggested by STROBE. In Table 2, age is modeled as a linear term. Please provide evidence that mortality increased according to linearity. **Discussion** There are many studies that have assessed combination therapy in patients with pneumococcal pneumonia. The authors should discuss their findings in relation to other studies that have focused on combination therapy in pneumococcal pneumonia not only to studies that have focused on therapy with macrolides in CAP. (Aspa et al Respir J 2006; Mufson MA et al Am J Med, Martinez JA et al Clin Infect Dis 2003; Baddour LM et al Am J Respir Crit Care Med 2004; Dwyer R et al Eur J Clin Microbiol Infect Dis 2006; Naucler et al Thorax 2013). In limitations the authors should address the problem of confounding by indication. The authors should also discuss how low sample size (outcome of only 21 deaths) might influence how step-wise backward selection process might influence results of multivariate modeling. Is this the best way to choose variables to include in the multivariate model? **<u>Specific Comments</u>** Row 9 page 4. Please specify country. Row 42-46 page 4. The authors state that surveillance studies show that S. pneumoniae is the 2nd and 3rd most common pathogens in pneumonia presented to the ED. There are regional differences in the microbiological panorama but surveillance studies are likely to result in misclassification since most patients are only partially tested against different pathogens. Some studies that have performed extensive microbiological testing have shown that *S. pneumoniae* is the most common pathogen in patients presenting to ED (e.g.

	Johansson et al CID 2009). Please include such references. Row 49 page 6. Please provide reference why 6 hours was chosen as cut-off for treatment to be regarded as appropriate. Row 17 page 9. This was not a trial but an observational study. Row 45 page 10. Do the authors refer to effect modification, i.e. interaction, or confounding. Please specify. Row 47 page 11. Please specify what is meant by ascertainment bias in this context. In table 1 provide explanations for abbreviations. In table 2, please specify what is meant by appropriate therapy.

REVIEWER	David Juurlink Head, Division of Clinical Pharmacology and Toxicology University of Toronto
REVIEW RETURNED	21-Apr-2013

THE STUDY	This is an observational study of the association between azithromycin therapy (in addition to another agent, generally a cephalosporin) and outcomes in patients with CAP or HCAP. The primary outcome is in-hospital mortality. This is an important topic that is not likely to be the subject of a RCT (see below). The effect size associated with azithromycin use is substantial, and I think the finding is clinically important, particularly in light of the gravity of S. pneumoniae infection and the low risk associated with azithromycin. The authors incorrectly describe the design as a cohort study. It is at best a case-control study. Table 1 describes individuals with the outcome and those without, these is no description of baseline characteristics at the outset of therapy, and the analysis employs logistic regression rather than, say, Cox proportional hazards analysis. The primary concern with a case-control study showing such a large

	effect size would be that selection bias or confounding explained the results. I doubt that is the case here - not just because of the magnitude of the effect, but because the expected direction of such a bias would oppose the effect seen here. In other words, if I preferentially added azithromycin to the regimens of sicker patients, I might expect to see azithromycin associated with an increased risk of poor outcomes. The opposite is seen here. I can think of no plausible reason why co-treatment with azithromycin would be preferentially avoided in sicker patients as the basis for the findings here. While it may be that other factors not balanced in Table 1 (esp. delay to treatment) partially explain the findings, I doubt they explain the strong and seemingly protective association seen with azithromycin. The statistical methods are rudimentary but this is likely necessary given the small sample at hand.
RESULTS & CONCLUSIONS	It's a fairly clear message with limitations as noted by the authors. External validation would be valuable. I am not sure I agree with the authors' call for a RCT, which might be difficult to justify in light of their findings. While it is probably premature to accept their findings as cause-effect, the effect size is large enough to throw equipoise into serious doubt. The outcome, after all, is mortality.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1.

1. We appreciate the reviewer's generally positive comments about our study.
2. The reviewer expresses concern about confounding by indication and suggests a propensity score be developed to help address this limitation. We agree that confounding by indication is always a concern with retrospective studies. However, in this case we feel a propensity score approach is inappropriate. First, the purpose of a propensity score is to essentially stratify patients into various cohorts of probabilities for receiving the therapy in question. From this a group of low probability to be treated patients (eg < 20%) can be compared to a cohort of high probability to be treated patients (eg >80%). This approach allows for a comparison of populations where they are segregated in a way to allow for something resembling randomization as it relates to confounding by indication. In our case, though, nearly 2/3rds of the cohort of subjects were exposed to macrolides. This precludes us splitting the population easily. Second, and related to the first point, our cohort is relatively small. With fewer than 200 patients, if we only compare a high to low probability of treatment population, the sample size will be significantly reduced so that we will be underpowered to explore anything of clinical relevance. Third, one would speculate if there were confounding by indication present in our cohort it would bias the data towards azithromycin not being associated with mortality. In other words, if physicians already thought there was a mortality benefit with the drug and were preferentially giving it to patients at high risk of death so as to gain some benefit then we actually should have seen macrolide use having no effect on mortality. In fact, we saw just the opposite. We have added language to the limitations section to note that confounding by indication is a concern with any retrospective analysis of this type.
3. The reviewer raises questions about our modeling approach. He suggests we explore utilizing CURB-65 score rather than MV or ICU admission. We appreciate the point that the exposure to the

macrolide might precede admission to the ICU and/or initiation of MV. However, either of these events occurs early in the course of severe CAP and CURB65 score necessarily is co-linear with need for MV or ICU admission. Nonetheless we have re-run the logistic regression utilizing CURB-65 score rather than either MV or ICU admission and now present the results as a sensitivity analysis. In addition (see below) we have utilized an enter approach rather than a stepwise regression for this sensitivity analyses in light of the low number of events (eg mortality). This sensitivity does not in any way alter our observation about the association between azithromycin exposure and mortality. We appreciate the reviewer's suggestion as it does appear to strengthen our conclusions.
4. We concur with the reviewer that a number of co-morbid illnesses are associated with mortality in pneumonia. Unfortunately, we lacked data regarding underlying pulmonary disease and liver disease. We now expressly note this in the limitations section.
5. The reviewer is correct that generally patients given moxifloxacin are not co-treated with azithromycin. In the present cohort, only 4 of 22 moxifloxacin treated also received azithromycin. Of the patients who did not receive azithromycin, the majority (43 of 61) were given a beta-lactam as monotherapy. In essence azithromycin exposure is co-linear with beta-lactam therapy. Therefore we agree with the reviewer that logically one cannot say the relationship is with azithromycin as opposed azithromycin combined with beta-lactam treatment. We have now added this concern the discussion of the paper.
6. All patients with pneumonia underwent full basic testing with blood and sputum cultures and urinary antigen testing. Patients unable to produce sputum spontaneously had cultures induced by respiratory therapy.
7. No isolate was in vitro resistant to the agent administered. We now clearly state this in the text.
8. With respect to Table 1 we have added the distribution of actual CURB-65 scores as well as the distribution of antibiotic selections. We prefer to keep this as one large table as breaking it into two tables would unnecessarily add to the length of the paper. We would point out that reviewer 2 has not asked us to do this.
9. For the results of the regression we now present both the original regression and the sensitivity analysis regression (with CURB-65 instead of either MV or ICU) and now, as the reviewer suggests, show the unadjusted ORs where appropriate.
10. The reviewer asks about the linearity of the relation between age and death. First, assumptions about linearity are an issue in linear regression, not logistic regression. Second, for logistic regression it is generally preferred to leave continuous variables as continuous rather than to falsely dichotomize them. Finally, when one breaks the age groups into quartiles (age 21-45, 46-55, 56-69, 70 and above) the mortality rates increase linearly (4.9%, 7.6%, 11.1%, and 21.0%, respectively)
11. The reviewer points out that there are other studies that we have not mentioned in the discussion that have examined this topic. We agree with the reviewer that there has been much attention focused on this topic. However, we respectfully disagree about the need to address the studies he notes. Most of these studies specifically address bacteremic pneumococcal pneumonia while we focused on all pneumococcal pneumonia patients, irrespective of presence or absence of bacteremia. In addition, most of the studies referred to are already included in several of the meta-analyses we cite and discuss. In light of these factors, the space constraints, and our broader focus than just bacteremia, we do not feel in necessary to exhaustively review the literature in this area.
12. Please see our comments above as it relates to confounding by indication. We concur with the reviewer and now note this issue in the limitations section.
13. The reviewer suggests that given our event rate that a stepwise approach may not be correct. We have reviewed this issue and concur that the reviewer is correct. As we note above, the regressions presented are now done via an enter approach. We appreciate the reviewer's statistical acumen on this point.
14. We now specify the country.
15. We appreciate the reviewer's point about the epidemiology of S. pneumoniae. We have revised the sentence and now cite the suggested reference.
16. We now cite a reference on this point of timing. The six hours was a typo and it should hve read

four hours as this represents the PN-5b quality measure that was enforced in the US for purposes of pay for performance by Medicare. Multiple studies have shown that in pneumonia, a delay in antibiotic administration is associated with death.
17. We have replaced the word trial as the reviewer requests.
18. We concur with the reviewer that the sentence is awkward. We have revised it. Our sole point here was to note that timing is a key determinant of outcome in this syndrome and most prior reports have not even attempted to address it or control for it.
19. Ascertainment bias refers to bias in determining an endpoint. For example, clinical cure may be a soft endpoint that can be biased in many ways. There is little difficulty in determining the patient's vital status at discharge. We have revised the sentence.

Reviewer 2.

1. We appreciate the reviewer's positive comments about our manuscript.
2. We respectfully disagree as to whether this is a case control or cohort study. In a case-control study cases are defined by the presence of an outcome. These cases are then matched to some controls that are chosen from similar underlying population but do not have the outcome. The exposure of interest is then explored backward from identified outcome. Because controls are generally selected rather than identified, the incidence or prevalence of the exposure cannot be calculated in a case-control study. Cohort studies, on the other hand, examine subjects from an a priori defined group (in our case, pneumonia) by the exposure (in our case macrolide treatment). Within this cohort subjects with the exposure are compared to those without the exposure with respect to the outcome of interest. This design allows for computing of incidence/prevalence of the exposure. In other words we can calculate the rate of mortality in those exposed to azithromycin and those not exposed. Therefore we have conducted a retrospective cohort study. We also respectfully disagree as to the description of baseline characteristics. We certainly present select baseline characteristics in Table 1.
3. We appreciate the reviewer's phrasing of the issues related to the potential for confounding by indication and have adopted some of them directly into the paper.
4. We concur with the reviewer that because of the retrospective design and the sample size we have likely over-estimated the treatment effect. We further concur and state in the paper that external validation is crucial.

VERSION 2 – REVIEW

REVIEWER	Pontus Naucler MD, PhD Dept of Microbiology, Tumor and Cell Biology, Karolinska Institute Sweden
REVIEW RETURNED	09-May-2013

RESULTS & CONCLUSIONS	The authors have addressed the issue of confounding effect of beta-lactam antibiotics on the association between azithromycin and mortality by a comment in the discussion. However, I believe this could be made clearer in the analysis section by including p-values for the association between different antibiotic classes and mortality in Table 1. For statistical power purposes Ceftriaxone, Cefipeme and Pip/Tazo could be combined. It seems that 17/21 (81%) of patients who died received a B-lactam antibiotics vs 135/166 (81%) of patients that survived which indicate no difference due to B-lactam antibiotics. However, if there are any differences for other antibiotic classes at a p-value <0.1 these should be included in the multivariate model. Also, exact figures for azithromycin therapy should be included in

	Table 1 under the section of Antibiotic therapy. In Table 1 I do not understand how 61.9% of patients who died vs. 91% who survived had delay in appropriate therapy and how this transforms into an odds ratio associated with improved survival for patients with appropriate therapy? Provide footnotes for abbreviations such as HD, LTC and MV in Table 1 and 2a. Please provide Unadjusted OR for age and CURB-65 score for table 2a and 2b.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1.

1. We have revised the table pooling beta-lactams and cephalosporins as suggested. The p value for the difference in this approach is essentially one. For no other antibiotic (other than azithromycin) is there a difference so there was no need to readdress the logistic regression.
2. The exact data for azithromycin is now shown in Table 1.
3. We appreciate the reviewer catching this error as it relates to rates of inapprop tx. In fact what was show were rates of APPROPRIATE therapy (the converse of what is in the text). We have corrected this now so that it is clear and makes more sense (and done this both in the table and the text).
4. We have added the footnotes requested.
5. We have added the unadjusted ORs as requested.