Author’s response to reviews

Title: Patient characteristics but not virulence factors discriminate between asymptomatic and symptomatic E. coli bacteriuria in the hospital

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Author’s response to reviews: see over
Dear Editor,

This letter is in response to the reviewers’ comments that you sent us on February 19, 2013. Below you will find point-to-point responses to their comments. All changes in the revised manuscript are highlighted in yellow.

Perhaps the most important aspect to point out is that we set up a prospective cohort of patients with *E. coli* bacteriuria to reflect the clinician’s evaluation for potential urinary tract infection. As a consequence, this “real-life sampling” included both patients with asymptomatic and symptomatic *E. coli* bacteriuria. Our objective was to identify characteristics that can distinguish these two groups and help with the clinician’s decision whether to start antibiotics or not. We hope we clarified this in the revision.

Thank you for considering the revised manuscript for publication in your journal.

Sincerely,

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Reviewer's reports and point-to-point response:

Reviewer 1

The Authors aimed to determine patient and pathogen factors suggestive of asymptomatic bacteriuria in a cohort of patients admitted to the same teaching hospital. They concluded that asymptomatic E. coli bacteriuria in hospitalized patients was frequent and more common in patients with dementia and chronic pulmonary disease. Moreover, bacterial virulence factors could not discriminate symptomatic from asymptomatic bacteriurias. Finally asymptomatic E. coli bacteriuria cannot be predicted by virulence screening.

Major Compulsory Revisions:
1) The data collection process is not clear. Did you use a prospective database or e-CRF?
We state in the first sentence in the methods section that “we performed a prospective cohort study…” Yes, the data that we analyzed for the purpose of this manuscript was prospectively collected. This is also mentioned in the abstract.

2) How did you assess the LUTS impact in your cohort? Did you use IPSS in male patients? The role of voiding LUTS is very important in ASB development.
We collected data from the medical records, including all patient-reported symptoms. Because our research team did not interact directly with the enrolled individuals we were not able to use questionnaires such as the international prostate symptom score (IPSS). The reviewer points to aspects of urinary symptoms that may not be documented well in medical records. We did, however, collect information on voiding problems (this information has been added to Table 1).

3) What are the inclusion and exclusion criteria?
In the first paragraph of the methods section we say that “all adult patients admitted to BJH who presented with E. coli bacteriuria at time of admission or developed it subsequently were considered for enrollment.” - these are the inclusion criteria (lines 82-83). Exclusion criteria were 1) polymicrobial infection, 2) concurrent non-E. coli bacteremia, and 3) inability of the patient to report urinary tract symptoms to the treating physician. These are listed in the methods section as well (all high-lighted in yellow).

Thanks for pointing out this reference. We added this article to the list of citations (line 59 in the introduction). Although we did not study outcomes the way the authors did (i.e., recurrence of infection over a long follow-up period) we determined that the majority, approximately 80% of ASB patients, were unnecessarily treated with antibiotics. It is very possible that this is detrimental in the long run, either by removing the protective impact of ASB against symptomatic infection or by increasing antimicrobial resistance. Another difference, which may have influenced treatment patterns is that Cai et al studied outpatients, while we focused on hospitalized patients.

Reviewer 2

The manuscript by Marschall et al. present two risk factors associated with asymptomatic E. coli bacteriuria in patients. Generally, the manuscript is well-written but the study design is not clear including exclusively one centre and some important experiments are not performed.

Major Compulsory Revisions
The manuscript has some major biases in the goal of the study:
-Ln 67-69 and Ln 204-206: The objective of the study was to develop a bedside test to distinguish ASB from UTI... I don’t understand the interest of this test. To distinguish ASB and UTI, you can use clinical signs... and except for some clear criteria (e.g. pregnancy...), no antibiotic treatment must be prescribed. So why would the authors develop a test? Please clarify.
The objective of this study was not to develop a bedside test but rather to understand how ASB (asymptomatic bacteriuria) can be differentiated from sUTI (symptomatic urinary tract infection) by objective criteria such as virulence factors and patient characteristics. It is correct, however, that a bedside test would be desirable (as made explicit in lines 67-68).
The reviewer asks why we would need an objective test when we can simply ask the patient about her/his symptoms. This is an important question. In reality, many ASB patients receive unnecessary antibiotic treatment in spite of being reportedly asymptomatic; also, for some patient groups such as confused or intubated patients it is simply not possible to obtain information on urinary tract symptoms and we have no guidance on whether to treat these patients (see added text in lines 68-71). It is our hope that in the future an objective test will be available to aid the provider in these difficult cases with the treatment decision-making.
-Moreover the study is monocentric and concerned a specific population (elderly patients). It’s very difficult to interpret the results and generalize these data in other Centres.
The reviewer is correct in that this is a single-center study and this was listed in the limitations. We clarified this limitation in line 268 (high-lighted). The difficulty to generalize to other centers was stated as well.
The reviewer is not correct, however, in thinking that we were only concerned with elderly patients. Table 1 reveals that the age range is from 19-101 years. Our study population included ALL adult patients admitted to the hospital who were also found to have E. coli bacteriuria.
-The recruitment of ASB and UTI is unclear. Which patients were screened? All the patients in the Center or some patients? What are the criteria the authors used to screen asymptomatic patients? Are prostatitis excluded? If so, please indicate and explain why. I don’t understand the strategy of blood cultures. All the patients with UTI and fever had not blood cultures and patients with ASB had blood cultures. Could you explain? One patient with ASB presented a blood culture + with E. coli: how the authors could excluded a pyelonephritis in this case?
As described in the methods section “we performed a prospective cohort study of patients with E. coli bacteriuria”. That is, the testing for urine pathogens depended entirely on clinician preference and was not mandated by the research team. We added a clarification in line 83/84 stating that urine culturing was done at the discretion of the treating physician. We included all adult patients with E. coli bacteriuria (except for a few exclusion criteria listed in the response to reviewer 1). Prostatitis was not excluded. Again, regarding drawing blood cultures this was dependent on diagnostic decision-making by the treating physicians (explained in lines 110/111). Our intention was to document practices regarding clinicians’ evaluation for potential urinary tract infections. Here, we found that providers obtained blood cultures for about 50% of their patients with symptoms; blood cultures, however, were also drawn in 30% of patients WITHOUT urinary symptoms or fever, which does not align with official recommendations. We think these blood cultures for asymptomatic patients are unnecessary, and our findings supported this supposition. The
single ASB patient with bacteremia could have had occult pyelonephritis since there was no documentation of fever, flank pain, or costovertebral angle tenderness.

-Some major virulence factors are not screened in this study (notably adhesins genes: papGII, papGIll, papA...). It’s well established that these genes are involved in UTI and could discriminate cystitis, pyelonephritis and ASB. See for example Lavigne JP et al., J Clin Microbiol 2011). The phylogenetic groups and serotyping are also very important in the analysis of E. coli virulence. These criteria are absent in this study. Moreover, the interpretation of the results is not surprising. Many publications showed that E. coli strains belonging to B2 or D phylotyping groups have a real potential of virulence and could induce UTI (Please see different articles on ASB and pregnancy). So the fact that no difference could be found between strains isolated from ASB and UTI is not surprising: the only difference is the genes expression. The authors must describe the phylotypes of the strains and perform clonality research (PFGE) to see if the strains are identical or not.

First, the reviewer is correct in that there are adhesin subtypes that we elected to summarize with genotyping for P-related fimbriae (abbreviated as “prf” and now better explained in line 120). This primer detects all the pap adhesins mentioned by the reviewer. We added to the limitations that we were restricted to testing a specific set of virulence genes (although more than most previous studies on that topic) and reference Lavigne et al’s paper there (on line 246).

A limitation of our study (as discussed in the manuscript, line 263) is that we did not carry out expression tests, though these are only practical for a few of the studied genes. We do not agree with the reviewer’s statement that “the fact that no difference could be found between strains isolated from ASB and UTI is not surprising: the only difference is the genes expression”. Other, smaller studies HAVE seen differences in the virulence factors (Mabbett et al, Takahashi et al). And to date it has not been proven that expression of virulence markers is truly what distinguishes ASB from sUTI.

Finally, our research budget prevented us from determining clonal relationships and serotyping our isolates.

Minor Essential Revisions
I have some others concerns about this study.
-Ln.30: Please indicate the location where the study was conducted.
We added this information (“at a tertiary care hospital in St. Louis, Missouri, USA”), please see line 31.
-Ln. 53: ASB screening is also recommended in immunocompromised patients. Although there may be controversy about who benefits from screening for ASB, the most recent IDSA guidelines DOES NOT recommend screening immunocompromised patients (Nicolle et al, Clin Infect Dis 2005;40:643-54).
-Ln 76-77: Data of nosocomial and community origins are mixed. This could induce a bias in data interpretation. Moreover the definition of community-acquired UTI is not correct. You must include in nosocomial infections all patients who had a link with healthcare system (e.g. Institution, Long-term care, etc).

The goal of this study was to examine bacteriurias in admitted patients; this automatically includes both patients with community-acquired and with hospital-acquired bacteriurias. It is true that this leads to a somewhat more diverse study population; however, this also provides a closer reflection of clinical practice.

Thank you for pointing out that the definition for community-acquired bacteriuria was incomplete. In fact, we identified patients transferred from outside hospitals or nursing
homes as “hospital-acquired” as well. We now address this on line 87 which now states: “Patients transferred from outside hospitals or long-term care facilities were not considered to have community-acquired bacteriuria”.

-Ln. 121-122 and Ln 170-173: The results must be presented in the Table 1 and discussed. The level of ciprofloxacin resistance is particularly high in this Centre. Please explain.

In response to this comment we added two lines of data to Table 1. We also noted the high prevalence of ciprofloxacin- and Bactrim-resistance on page 15, line 269.

-Ln. 137-139: The authors must record the patients’ signature.

As we obtained IRB approval with a waiver of informed consent, patients’ signatures were not required.

-Ln. 143: I strongly recommend that the authors add a flowchart.

For brevity, we decided to list the total number of enrolled patients and then give the respective numbers for symptomatic (n=177) and asymptomatic (n=110) patients. If the editor prefers a flowchart, we will be happy to provide it.

-Ln. 159: “patients with symptomatic UTI were more likely to be neutropenic”. It would be interesting to know the leukocyturia in urines samples of these patients. The detection of this criterion is very easy (e.g. dipstick), low cost and could represent a solution in this population (if I follow the goal of this study).

We collected this information and presented it on Table 1 (page 26, bottom). Leukocyturia >10 cells/high power field did not distinguish between ASB and sUTI, unfortunately.

-Ln 214-215: The link between chronic pulmonary disease and ASB could be exclusively due to a bias in the recruitment (with an elderly population which presented this comorbidity).

Again, we did not focus on geriatric patients but included adult patients of all ages. However, the reviewer is correct that chronic pulmonary disease could be a random association that occurred due to urine culturing practices specific to certain medical subspecialties (mentioned in line 229-231).

-The references are not updated. To improve discussion, please see for example: Ragnarsdottir B et al., Pediatr Nephrol 2012 (the authors observed some virulence factors and host markers which are involved in risk factors of UTI or ASB); Hawn TR et al., PLoS One 2009 (the authors show a relation between genetic factors and UTI/ASB – in response to Ln 246-248); Ariathianto Y, Aust Fam Physician 2011 (the author identified dementia as a risk factor of ASB – in response to Ln 213); Juthani-Mehta M, Clin Geriatr Med 2007 (the author wrote a review on ASB and UTI in elderly population).

Thank you for suggesting these references. We included the very interesting article by Hawn et al into the references for our statement that the innate immune system may play a crucial role in determining who develops ASB (line 256). Also, Ariathianto is now cited to make the point that cognitive changes are linked to ASB (line 226/227). We did not include Ragnarsdottir et al, because theirs is a review article focusing on pediatric patients. We also did not include Manisha Juthani-Mehta’s article focusing on elderly patients because it is a review and not original data.

Reviewer 3

This is a prospective cohort study of patients with E. coli bacteriuria from August 1, 2009 to July 31, 2010 at a 1250-bed teaching hospital. All adult patients admitted to the hospital that presented with E. coli bacteriuria at time of admission, or developed it subsequently were considered for enrolment. The
study analyzed patient and pathogen factors associated with asymptomatic versus symptomatic E. coli bacteriuria. The study concludes that asymptomatic E. coli bacteriuria in hospitalized patients was frequent and more common in patients with dementia and chronic pulmonary disease. Bacterial virulence factors could not discriminate symptomatic from asymptomatic bacteriuria.

Major compulsory revisions
Those classified as asymptomatic bacteriuria (ASB) in the study had no symptoms of cystitis and no symptoms of pyelonephritis. Why had the clinician ordered a urinary culture? It might be a selection bias in this prospective study as urine cultures were taken at the discretion of treating physicians and not from everyone admitted to the hospital. If a urine culture had been taken from everyone admitted to the hospital, there would have been many more patients with ASB. It’s not unlikely that patient characteristics among those patients could be different compared to patients where the clinician for some reason took a urine culture in the absence of urinary tract symptoms.

The reviewer poses an important question: Why are urine cultures obtained? We believe (but cannot prove) that many urine cultures are not indicated, and may detect asymptomatic bacteriuria which is then often treated unnecessarily. The reviewer is correct that there could be selection bias. We mention this in the limitations (lines 259/260). Unfortunately, we were not funded to obtain urine cultures for the entire hospital population. But this should be done in a future study and might easily yield more ASB cases as the reviewer points out.

It is also conceivable that we identified a specific subset of screened ASB patients who differ from others with ASB who are less likely to be screened. In this way, the patient characteristics we identified could have revealed which patients are BOTH asymptomatic and also at risk for being unnecessarily worked-up with urine cultures (this possible bias is stated in lines 260-262).

During the entire year, there were only 70 patients with cystitis symptoms and E. coli in the urine. This seems to be a small number at a 1250-bed hospital. (Totally there were 337 patients with E. coli bacteriuria, 50 were excluded as they were considered unclassifiable, 110 had ASB, 107 had pyelonephritis and 70 had cystitis.) Why are these numbers so low? Are there other urine cultures taken at the hospital, but not included in this prospective study? Were treating physicians just taking urine cultures sporadically when patients presented with cystitis symptoms? Either of these explanations could affect the selection of studied subjects in a way that could possibly affect the results of the study.

Yes, we present monomicrobial E. coli bacteriurias in adult patients admitted to our hospital during one year. The only exclusion criteria applied to these patients were 1) concurrent non-E. coli bacteremia because it could easily confound the clinical presentation; but this was a small number of patients, and 2) those who could not report symptoms (i.e., those 50 with “unclassified bacteriuria”) (mentioned in lines 151/152). There were no other cultures taken in hospital patients. We therefore do not believe that there was additional bias introduced beyond what we discuss in the response above. Furthermore, we believe that urinary symptoms serve as the main indication for obtaining urine cultures at our institution.

According to methods, presence of dysuria, frequency, urinary retention or lower abdominal pain was classified as cystitis symptoms. There is no information on time aspects and number of symptom required for being classified as having cystitis. Especially among elderly people there are many other causes to these symptoms, especially if not of recent onset, i.e. prostatic hyperplasia and
genitor-urinary symptoms associated with oestrogen-deficiency states. Depending on which definition used, patients with ASB could be misclassified as cystitis if having urinary symptoms caused by something other than E. coli in the urine.

We identified E. coli bacteriuria together with at least one urinary symptom of the above as cystitis, according to expert recommendations for diagnosing UTIs (Hooton, New Engl J Med 2012;366:1028). We do not believe that ASB patients were misclassified as having cystitis.

There is no information about the number of urine samples cultured during the year of the study. What was the distribution between urine cultures positive/negative for E. coli?

Unfortunately, we do not have access to this information.

Is it possible that single patients could be included more than once in the study, if readmitted to the hospital?

Yes, but this was an uncommon occurrence (n=7).

3. Are the data sound?

It is not stated how patients were selected for a urine sample. Due to possible selection bias, the comparison between symptomatic and asymptomatic patients with E. coli could rather be a comparison between the following two groups; E. coli bacteriuric patients with UTI symptoms and a complicated group of patients that the clinicians didn’t know how to handle (as they took urine cultures in the absence of UTI symptoms).

This is an important remark and we now include this thought in the discussion (line 230/231). We agree with the reviewer that these “complicated…patients that the clinicians didn’t know how to handle” in fact are the clinically relevant ASB patients because for some reason, they had urine cultures drawn that became positive, and now the clinician has to decide what to do about these E. coli bacteriurias in asymptomatic patients (that he/she shouldn’t have screened for to begin with). If we did surveillance for all ASBs this would be a different, pathophysiologic study; here, however, we essentially compare clinically relevant ASBs to symptomatic UTIs.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

Yes.

5. Are the discussion and conclusions well balanced and adequately supported by the data?

Due to possible selection biases and a difficulty to define a true UTI versus ASB, the conclusions might be incorrect.

This comment has already been answered above, in response to reviewer 2.

6. Are limitations of the work clearly stated?

The possible selection biases (mentioned above) are not clearly stated in the manuscript. Other limitations of the work are however clearly stated.

We made this potential for bias more explicit (line 259).

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?

Yes.

8. Do the title and abstract accurately convey what has been found?

Yes.

Reviewer 4

Major Compulsory Revisions:
This study addresses an important problem - that is to differ between ASB and sUTI when finding bacteriuria. However, there are some problems when defining ASB:

First there is the case where the patient is not able to report urinary symptoms (a demented or seriously ill patient). In this study I understand that these individuals were excluded. **Correct, these individuals were excluded.**

The second group constitutes of patients with no distinct urinary symptoms but with more general symptoms that leads the doctor to suspect a sUTI. Here the evidence of benefit of antibiotic treatment is low and most cases could probably be classified as ASB. **Correct, if there were no distinct urinary symptoms, these patients were considered to have ASB.**

The third group could represent those patients where there is no suspicion of a sUTI but a urinary was yet taken (you could say by mistake). **Yes, as indicated in response to a comment from reviewer 3 we think that this may represent unnecessary care (see also added text in lines 230/231).**

1. You should better clarify, in the method, in which patients there was a culture obtained not just state “who presented with bacteriuria”. Are they from the second and/or third group mentioned above? Was there a culture obtained from all patients or from those with some kind of symptom?

As discussed in response to an earlier comment and now added to the methods section, it was the providers’ discretion to obtain urine cultures. As far as we can tell from the included adult patients who had E. coli bacteriuria: there were many patients without urinary symptoms or fever in whom the indication for urine culturing is questionable in hindsight.

2. You should take this more in account in the discussion, because the way you recruit patients for a urinary culture affects the ability to interpret the results in terms of risk factors – it could be that meeting a patient with some possible risk factor could give you an impulse to obtain a urinary culture.

In fact, only a minority of patients in this cohort had ASB that resulted in a treatment indication (i.e., pregnant patients and those about to undergo urogenital surgery).

**Minor Essential Revisions:**

“Complicated bacteriuria” – what is that?

The concept of “complicated UTI” has been introduced several decades ago to denote a patient with a more complex history (such as being immunocompromised, male, or being diabetic). We applied the same concept to bacteriurias irrespective of symptoms, hence “complicated bacteriuria”.

**Discretionary Revisions**

If there was a culture taken in all patients - is it possible to get information on the total number of patients to relate to the number of bacteriurias? **Unfortunately, we do not have access to this information.**

What about those with bacteriuria but with other bacteria than E coli? How big was this group?

We focused on E. coli because these are the predominant uropathogens in community as well as in hospital-acquired UTIs. We did not collected information on any other pathogens.