Author's response to reviews

Title: Antibiograms from community-acquired uropathogens in Gulu, northern Uganda - a cross-sectional study

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

Below, we present responses to comments raised by reviewers of our pending article titled: Antibiograms from community-acquired uropathogens in Gulu, northern Uganda - a cross-sectional study. We have presented a point by point response to each comment directly to the reviewer. All responses are presented in the order in which they are raised.

Thank you for providing us ample time to make these responses.

Yours faithfully,

Charles

(REVIEWER 1: CASPER DEN HEIJER)

Major Compulsory Revisions

Introduction

1) The last six lines in the second paragraph of the introduction section have been rewritten to clearly bring out the broad and specific aims of this study. The broad aim was;

   • To examine the validity of the current recommendations for treatment of community-acquired UTI as per the Uganda Clinical Guidelines

The specific aims were;

   • To identify uropathogens from community-acquired infections at Gulu regional hospital
   • To determine the resistance profiles of uropathogens against antibiotics recommended for empirical treatment of community-acquired UTI in our setting.
   • To determine the resistance profiles against a range of potentially useful alternatives for treatment of community-acquired UTI in our setting.

Methods

2) We carefully thought through the use of leucocyturia as a criterion for true UTI determination. We agree with your observation that other conditions, in addition to UTI may indeed cause leucocyturia e.g. urinary Schistosomiasis, which is not uncommon in our setting. To address this problem, we adopted a two-stage screening approach before confirming each suspected sample as ‘true UTI’. First, all urine samples from
participants who had symptoms were subjected to a leucocyte count. Secondly, all samples that showed significant leucocyturia were subjected to quantitative culture before a decision of 'true UTI' was reached. In some cases, growth of a single organism regardless of cut-off value, associated with recent history of antibiotic use (prior to presenting at the hospital) was considered UTI. Indeed, as shown in table 1, there were 115 female participants whose urine had significant leucocyturia. However, only 64 of these had significant cultures. An additional 15 samples (making a total of 79) were finally considered true UTI basing on urine culture and antibiotic use-history. Leucocyturia with negative cultures were considered of a non-UTI origin. We have re-written the third paragraph in the methods section (Determination of true infection and identification of uropathogens) to clarify on this approach. In addition, footnotes to table 1 have been added for further clarity.

3) In our setting, it is particularly challenging to rely on the accuracy of participant responses as recorded on questionnaires. This is so because significant proportions of our participants are illiterate and will not distinguish between analgesic and antibiotic use. Unfortunately, these are the most common drugs purchased from outlets without prescription. Even with the benefit of questionnaires, we could not conclude with certainty that an antibiotic (and not an analgesic) was used prior to presenting at the hospital. We therefore felt more comfortable subjecting all urine samples to a leucocyte count which was feasible and cost effective compared to subjecting all suspected samples to quantitative culture from the onset. We felt that this approach increased the sensitivity of our ‘true UTI’ recruitment strategy. Thereafter, quantitative urine culture enabled us to exclude non-UTI cases of leucocyturia.

4) This study aimed to provide a general picture of the nature of community-acquired uropathogens and their resistance profiles at Gulu regional hospital. As such, all true UTI cases presenting at out-patient units were included. In the first paragraph of the results’ section, we have described the general participant characteristics likely to influence categorization of UTI. Even then, only three males were recorded (3/82). We initially felt that separate analysis of such a low number would not be meaningful. Nevertheless, we have now included an additional table (table 4) with specific information regarding the uropathogens and resistance profiles from male patients.

With regard to grouping pregnant and non-pregnant women together, our national treatment guideline does not distinguish between empirical treatment of UTI in these groups (except for fetal safety considerations). It is within this context that the two were grouped together.
It is also true that the study population is predominantly young (with a mean age of 23 years for females). This is a reflection of the general Ugandan population where 77% are estimated to be less than 30 years of age (source: Uganda Demographics Profile, 2012. www.indexmundi.com/uganda/demographics_profile.html).

Minor essential revisions

General comments

5) We have changed all the capital letters on antibiotic names to small letters throughout the manuscript (e.g. gentamicin, amoxicillin, ciprofloxacin etc.)

6) The name ‘gentamycin’ in the methods section has been corrected to read gentamicin. This is now consistent with the rest of the text.

Methods

7) The cut-off value of $10^5$ CFU/mL was adopted for both male and female participants (including pregnant women). This is consistent with standard recommendations (see reference no. 24 in manuscript). However, in the case of participants with a recent history of antibiotic use, we did not strictly apply this cut-off value. Instead, a combination of findings including leucocyturia plus growth of a single organism on urine culture was considered sufficient. However, these findings had to occur in the absence of other abnormalities such as haematuria or presence of parasitic ova on urine microscopy. As reported in table 1, a total of 67 patients had $10^5$ CFU/mL on quantitative culture. This means that decision on an additional 15 patients (to make a final total of 82) was based on a combination of other findings including leucocyturia, quantitative culture, purity of growth, recent history of antibiotic use etc.

8) Clinicians were asked to refer (to the study team) all patients who had any of the symptoms that were likely to suggest a UTI. These included supra-pubic pain, burning sensation while voiding (dysuria), increased urine frequency and urge incontinence. We have re-written the first paragraph in the methods section (under study design) to clearly bring this out. Although these symptoms are not specific to UTI, they nevertheless provided a screening point from which we were able to identify cases that were considered ‘true UTI’ using a two-stage urine investigation approach. All findings were returned to clinicians in a timely manner so as to inform appropriate care.
Results:

9) There were indeed few instances were more than one organism were isolated on quantitative culture. However, in all such cases, none of the organism reached the set level of significance (i.e. $10^5$ CFU/mL). In some cases, isolation of more than one organism was accompanied by absence of leucocyturia. Such cases were considered contaminants and discarded without further investigation. However, with the benefit of hindsight, we acknowledge that some cases of polymicrobial UTI may have been incorrectly omitted from the study. In the last paragraph under the discussion section, this has been acknowledged as a possible limitation in this study.

10) We do not think that the finding of Staphylococcal species as the dominant uropathogens was due to a selection of patients. Indeed Escherichia coli still featured prominently in this study, although the situation was slightly different from what is commonly observed elsewhere. A Ugandan study similar to ours (reference no. 14 in manuscript) found Staphylococcal species (31.9%) and E. coli (10.1%) as the dominant uropathogens at a metropolitan hospital in Kampala. We are of the view that the predominantly young participant population seen in our setting is responsible for this etiological pattern. Staphylococcus saprophyticus an organism known to reside in the urinary tract of sexually active women (Rupp et al., 1992) was the predominant organism isolated among staphylococcal species. It has also been implicated as a common uropathogen in this group (Jordan et al., 1980). We think that our results should not be surprising considering that majority of our participants were young women, presumed to be in active heterosexual relationships. This statement is implicitly supported by the fact that Uganda has the second highest fertility rate in the world after Yemen [source: www.populationaction.org]). We allude to this view in our discussion section.

11) We appreciate this suggestion. Confidence intervals at 95% level of significance have been added to all the values in table 3.

12) The use of nitrofurantoin discs of different potency (300 µg in Mwaka et al., and 50 µg in ours) was a critical difference between the two studies. These were adopted from different performance standards references and may explain the wide disparity in results presented.
In addition, the study by Mwaka et al., was done in Kampala, located in the south of Uganda. This is a different geographical region located about 400 kilometers away from Gulu in the north. Kampala and Gulu are predominantly urban and rural settings.
respectively. In addition, significant socio-demographic differences exist between the two populations in terms of occupations, lifestyles, cultures, attitudes and literacy levels. All these factors influence health behavior and may eventually determine antibiotic susceptibility differences over time. These views have been captured in the third paragraph of our discussion section.

**Discussion:**

13) We have re-written the discussion section to shorten it accordingly. Some of the results that were previously in narrative have been transferred to an expanded table 1.

14) The term ‘molecules’ has been discarded in favor of ‘antibiotics’ as suggested.

15) The information on the different species of Staphylococcus has been added in the second paragraph of the results section as suggested.

16) We absolutely agree and share your concerns regarding the toxicity of gentamicin and therefore its possible choice as empirical treatment of community-acquired UTI. However, we feel that our recommendations remain a realistic option for our setting. Our position is informed by awareness of the resource constraints within the public health care system in which we practice. Only 8% of the national budget is allocated to the entire health sector. Such under-funding certainly makes it impossible to provide safer but probably more expensive alternatives (e.g. amoxicillin-clavulanate, ceftriaxone, fosfomycin etc) to all who need them. We worry that within the current circumstances, recommendations far from what is feasible are likely to remain on the bookshelves and have no impact on the status quo. However, with respect to the toxicity concerns associated with aminoglycosides, our hospitals have adopted the newer once daily dosing strategy for gentamicin. This has been shown to be safer than the traditional multiple dosing strategy employed the past (see references 3-5 below). The fourth paragraph in the discussion section has been re-written to include these issues.

17) The strength and weaknesses of the study have been explored in the first part of paragraph six of the discussion section.
Tables:

18) As presented at the beginning of the results section, out of 339 urine samples collected, only 118 had significant leucocyturia. Since this criterion alone is not conclusive for UTI, quantitative urine culture (with $10^5$ CFU/mL cut-off value) was done on the 118 samples. By this two-stage criteria, we were able to include only 82 cases of true UTI. 36 samples that showed leucocyturia but non-significant growth on quantitative culture were discarded. Footnotes to table 1 have been added to clarify these figures.

Discretionary revisions:

Tables:

19) The category of patients with true UTI has been well defined in this study. However, we find the second category (i.e. patients without true UTI) a little vague because it includes all the rest (even those with supra pubic pain but negative cultures and those without urine leucocytes). Therefore, data from this category is not easy to summarize in a meaningful way.

References:

Dear reviewer,

We have numbered and responded to each question in the order in which they are raised. Each question is answered in a separate paragraph.

1. Before adopting this urine collection protocol, we reviewed literature relating to urine collection in female research participants. However, it was clear that this was not an easy procedure if it is to be free of contamination. Jaffe JS [1], in a letter to a journal editor summed it all up as follows: “The practical difficulty of collecting uncontaminated urine samples from women is well known. This is true even for the most educated of women, such as nurses”. Our decision to wipe the labia minora with clean gauze dipped in normal saline was aimed at minimizing contamination as much as possible. However, recognizing that the procedure was likely to be of a sensitive and uncomfortable nature, we employed trained nurses who shared a similar cultural background as the participants. These nurses were required to be fluent in the local language of the participants. We felt that this would enable effective communication which was important to promote comfort foster co-operation of the participant.

2. We are of the view that the small number of true UTI cases arose out of the stringent criteria for labeling a suspected case as UTI since neither leucocyturia nor quantitative culture was sufficient to confirm UTI in all cases. We used a two-stage screening approach to determine true UTI cases. In the first stage, 115 out of 339 urine samples were found to have significant leucocyturia. In the second stage, these (115) were subjected to quantitative culture and only 67 samples yielded significant growth. Furthermore, even if they did not yield significant bacterial growth, an additional 15 samples were included as true UTI (total 82) because a positive history of antibiotic use prior to visiting the hospital was established (using the standard form/questionnaire).

3. In total 69 participants (20.4%) had consumed antibiotics prior to visiting the hospital although most turned out to have a non-significant leucocyturia. This has been included in the first paragraph of the results’ section. Regrettably, all suspected cases where more than one organism was isolated were considered contaminants and therefore discarded. These stringent criteria might have led to the very low numbers observed in this study. We have re-written the last paragraph of the discussion section to include these possible limitations in our approach.
4. We did not collect any information on vaginal washing. Evidence from our 
questionnaires suggests that all participants were of a heterosexual orientation. In our 
discussion section, we propose that the high proportion of true UTI caused by Staph. 
saprophyticus may be explained by the known association between this organism and 
frequent (hetero) sexual activity among women. We think that the youthfulness of our 
study population together with the high fertility rates among Ugandan women implicitly 
support this view [2-4], equivalent to new references 36, 37, 38 in manuscript text. This 
view is echoed in the fifth paragraph of the discussion section.

5. This section has been re-written for better clarity.

6. Thank you for the comment and suggestion. In view of the low numbers, we feel that 
not much will be realized from either univariate or multivariate analysis at this point. 
We however intend to merge this dataset with that from an on-going study in order to 
enable this kind of analysis at a later stage.

References:

   (9): 617-618.
   Staphylococcus saprophyticus. Journal of Clinical Microbiology; 30 (11): 2975-2979
   Staphylococcus saprophyticus. Journal of Infectious Diseases; 142 (4): 510-515
4. Uganda total fertility rate: total fertility rank chart. [www.indexmundi.com/uganda/total-