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Isolated Bacterial Uropathogens among Pregnant Women Diagnosed with Asymptomatic Bacteriuria in Harare, Zimbabwe

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Authors' contributions

This work was carried out in collaboration between all authors. Author MRJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NP and FGM managed the analyses of the study. Authors OHC and SPB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to identify bacterial uropathogens responsible for asymptomatic bacteriuria among pregnant women registering for antenatal care at selected clinics in Harare, Zimbabwe.
Study Design: A cross sectional study design was conducted at 4 selected primary health care centres in Harare in Zimbabwe. The study period stretched for 18 weeks from 23 March to 27 June 2017.
Methodology: Mid- stream urine samples for 240 pregnant women registering and visiting 4 selected clinics for antenatal care were examined for asymptomatic bacteriuria. Griess nitrate test

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was used to screen the samples. All samples that grew significant bacteriuria were further tested by culture test. Uropathogens isolated from urine samples which had significant growth were identified by using morphology, gram stain and several biochemical tests.
Results: Out of 240 urine samples examined, 34 were significant for asymptomatic bacteriuria. Ten bacteria strains were identified. *Coagulase negative staphylococcus* was the most popular (29.4%) followed by *Escherichia coli* (23.5%). The identified species also included *staphylococcus aureus* (11.8%), *Klebsiella pneumoniae* (8.8%), and *Bacillus* (8.8%).
Conclusion: A variety of bacterial uropathogens both gram negative and gram positive, are responsible for asymptomatic bacteriuria. *Coagulase negative staphylococcus* was the most commonly identified bacteria followed by *Escherichia coli*.

Keywords: Bacteria; uropathogen; pregnant; asymptomatic bacteriuria; bacterial colony; significant bacteriuria.

ABBREVIATIONS

ASB : Asymptomatic bacteriuria
UTI : Urinary tract infection
CoNS: Coagulase negative staphylococcus

1. INTRODUCTION

Asymptomatic bacteriuria (ASB) is an occult infection in which significant actively multiplying same bacterial strain grows in urine sample obtained from an individual without urinary tract symptoms [1,2]. The condition is generally said to be significant when a culture of freshly voided urine reveals growth of 10^5 colony forming units per millilitre ($>10^5$ cfu/ml) or more [3]. The disease is common during pregnancy with prevalence generally ranging from 2% to 10% [4,5].

There are several changes occurring in pregnancy that result in an increase in risk of the infection in pregnancy [2]. Anatomic, physiological hormonal and biochemical changes that occur in pregnancy increase the risk of the pregnant women having active proliferation of bacteria in urine to significant levels resulting in ASB [5,6]. The reduced immunity in pregnancy and biochemical changes occurring in pregnancy promote growth and multiplication of commensals and non- commensal bacteria in the urinary tract [6,7]. The proximity of the already short urethra to the vagina which is usually colonised by bacteria from the GIT make it easy to transfer the microorganisms to the urinary tract [5,7].

UTIs are caused by a variety of pathogens including bacteria, fungi, viruses and helminths [8]. ASB infections in pregnancy are caused by bacterial uropathogens, as they are for the other UTIs across all ages [8]. More than 70% of these bacterial uropathogens are gram negative bacteria [9]. The bacteria often originate from the gastrointestinal tract [7]. *Escherichia coli (E. coli)* has for several decades been the most common uropathogen isolated, responsible for more than 80% of cases [1,7,10]. The other species include *Klebsiella, Proteus, Enterococcus species*, *Pseudomonas* and gram positive including *Coagulase negative staphylococcus* [8,11,12]. *Staphylococcus aureus* is another bacterial uropathogen commonly isolated among women diagnosed with ASB [13].

ASB often progresses later in pregnancy to symptomatic bacteriuria urinary tract infections (UTIs) including cystitis (40%) and pyelonephritis (30%) [10,12]. The resultant symptomatic urinary tract infections are associated with maternal and neonatal mortality and morbidity and adverse pregnancy outcomes including preterm birth, intrauterine growth retardation, and anaemia and thrombocytopenia [7]. Proper diagnosis of ASB is essential to ensure all the infected women are treated to reduce risk of developing pyelonephritis which is associated with adverse pregnancy outcomes [13].

The etiological agents and pattern of disease causation varies from setting to setting [12]. Knowledge of etiological agents for ASB is important for determination treatment strategy in order to prevent disease progression, relapse and reinfection which are very likely in pregnancy [7]. When bacterial invasion occurs in urine during pregnancy, endotoxins released by the uropathogens result in local circulatory disturbances in placenta which often leads to abortions, still birth intrauterine growth retardation, preterm birth and low birth weight [5, 10]. The aim of this study was to identify bacterial uropathogens responsible for asymptomatic bacteriuria among pregnant women.

2. MATERIALS AND METHODS

2.1 Design, Setting and Ethical Approval

A descriptive cross sectional study was conducted at 4 primary health care centres in Harare for 18 weeks which extended from 23 February to 27 June 2017.

2.2 Sample Size and Sampling Method

Sample size was determined by a calculation for the main study from which the objective of this study was created. The sample size was calculated using the Dobson formula $n = (Z\alpha + Z\beta)^2 [P1 (1-P1) + P2 (1-P2)] (P1 - P2)^2$. A minimum of 190 participants were required. The simple random sampling method was used on recruitment.

2.3 Inclusion and Exclusion Criteria

Pregnant women visiting the selected sites for registration or follow up visit for antenatal care with a gestation between 6 and 22 weeks who declared no urinary tract symptoms and with no knowledge of urinary tract deformity were included in this study. Those unaware of their last menstrual period dates and unwell or with urinary tract deformity were excluded in this study.

2.4 Ethical Considerations and Approval

All potential participants recruited were required to sign a consent form before participation commenced. Participants refusing to sign were not penalised. Confidentiality was assured as no name was attached to document s with information about the patient. Serial numbers were used for identification. Ethical approval for this study was granted by Medical research council of Zimbabwe, approval number MRCZ/A/2102.

2.5 Specimen Collection

Instructions were given to participants with special emphasis on prevention of contamination and submission of the collect clean catch sample. Cleaning of perineum was discouraged. Ten to 20 millilitres of mid- stream urine was collected into clean labelled specimen jars and submitted for screening for asymptomatic bacteriuria.

2.6 Screening Test and Screening for Asymptomatic Bacteriuria

Griess nitrate test and culture test. All submitted urine samples were screened at the study site for asymptomatic bacteriuria by Griess nitrate test within 30 minutes from collection time. The Griess test, a nitrite detection test, involves adding singly 2 reagents namely Sulfanilamide and N- 1- naphthylethylenediamine dehydroxide, in a space of a minimum of 5 minutes. A positive sample was identified by a colour change from clear to purple whilst a negative sample remained clear.

2.7 Asymptomatic Bacteriuria Diagnosis

All samples that changed colour were stored in a cooler box with frozen ice packs and transported to a laboratory at the Medical Microbiology Department of the University of Zimbabwe for culture test. As soon as the samples were received the laboratory scientist inoculated the samples uncentrifuged on Blood and Cystine lactose electrolyte deficient agar. A medium that grew a significant amount of bacteria was considered positive for ASB. In this study bacterial colony levels of above 1000 per millilitre ($\geq 10^3$ cfu/ml) were considered significant for ASB. A culture that yielded mixed insignificant ($<10^3$ cfu/ml) growth was considered contaminated [14].

2.8 Bacterial Uropathogen Identification

The plates with significant growth were included for identification process. Bacterial identification was done considering bacterial colony morphology, gram stain and biochemical tests. Gram stain was done to separated gram negative and gram positive. Several biochemical tests were done on gram negative to identify the specific bacteria causing ASB. The tests included Kligler Iron Aga test, motility test, indole tests, carbohydrate fermentation and citrate tests. Catalase test was used for the gram positive bacteria to differentiate staphylococcus and streptococcus, followed by biochemical tests [14].

2.9 Data Analysis

Data was entered on a spread sheet of IBM Statistical Package for Social Sciences program, version 20 for analysis. Data was also exported to a Microsoft Excel page for further analysis using STATA version 13. Simple descriptive statistics were used to describe patient's demographic characteristics of participants.

3. RESULTS

3.1 Demographic Characteristics of Participants

The ages of the women ranged from 15 to 41, with a mean of 25.6 years. Gestation of the women was between $6^{2/7}$ weeks and maximum of 22 weeks. Majority of the women were nulliparous (37.9%), and single parity (37.1%). Only 6 (2.5%) women went up to tertiary level whilst majority (192, 80%) had ordinary level of education. Most of the women were unemployed (170, 71%) with only 15 (6.2%) formally employed.

3.2 Screening and Diagnostic Test Results

Fifty samples were considered positive from the Griess test. These were tested by culture test. Six (12%) samples had no bacterial growth, 9(18%) were contaminated, 1 (2%) had non-bacterial, yeast growth as presented on Table 1. Out of the 50 samples tested by culture 34 (68%) were significant for ASB.

Table 1. Culture test results

Culture results	Frequency	Percentage (%)
Significant growth	34	68
No growth	6	12
Contaminated	9	18
Non- bacterial growth	1	2
Total	50	100

*Diagnosis of asymptomatic bacteriuria from significant growth

Out of 240 women who participated in this study 34 had significant bacterial growth and diagnosed ASB. The prevalence of ASB in this study was 14.2% (95% CI, 10.28% to 19.22%). Fig. 1 illustrates the process of ASB case and bacterial strain identification.

3.3 Isolated Bacterial Uropathogens

A total of 10 bacterial species were identified in this study. The most popular isolates belonged to the *Coagulase negative staphylococcus (CoNS)* (29.4%), followed by *Escherichia coli (E. coli)* (23.5%), *Staphylococcus aureus* (11.8%) and *Klebsiella pneumoniae* (8.8%). Table 2 shows the list of these and the other identified bacterial uropathogens in this study.

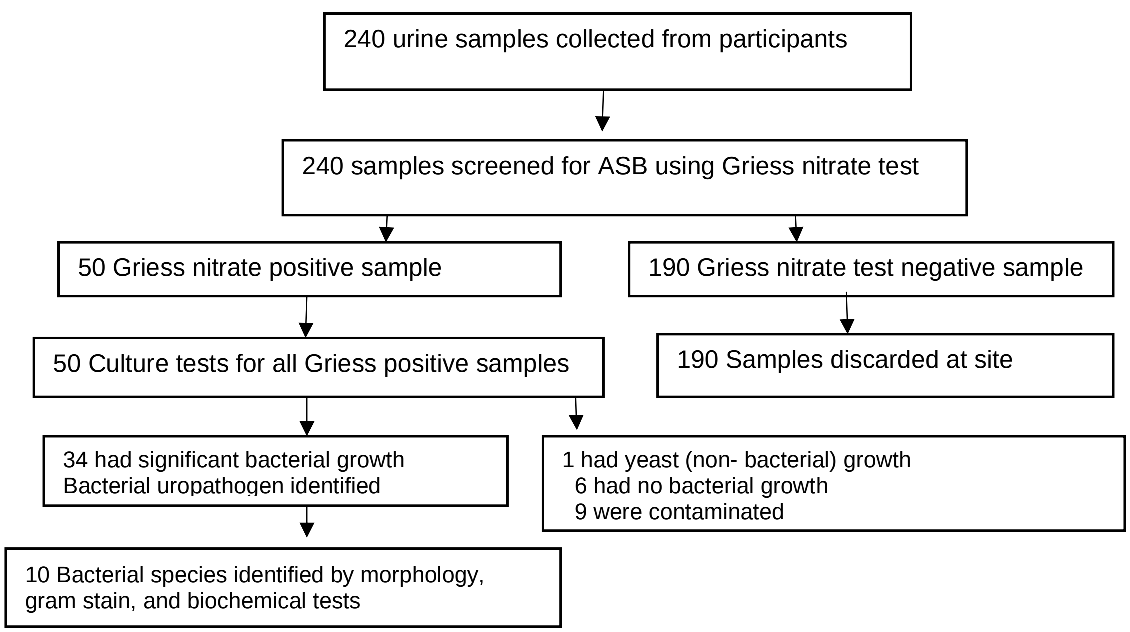

Fig. 1. Asymptomatic bacteriuria diagnosis and bacterial uropathogens identification process
*Process and results on identification of asymptomatic bacteriuria and bacterial isolates

Table 2. Isolated bacterial uropathogens

Number	Bacteria strain	Frequency	Percentage
1	*Coagulase negative Staphylococcus*	10	29.4
2	*Escherichia coli*	8	23.5
3	*Staphylococcus aureus*	4	11.8
4	*Klebsiella pneumonia*	3	8.8
5	*Bacillus species*	3	8.8
6	*Group D streptococcus*	2	5.9
7	*Salmonella species*	1	2.9
8	*Providencia*	1	2.9
9	*Streptococcus viridans*	1	2.9
10	*Shigella species*	1	2.9

** Identified bacterial species responsible for asymptomatic bacteriuria among pregnant women*

4. DISCUSSION

ASB is a common UTI in pregnancy. Although the prevalence of ASB is commonly ranging from 2% to 10%, it varies from setting to setting, with higher rates above 35% in the developing world [4,5]. A critical factor to the different rates could be explained by the different sample sizes for different studies. Demographic characteristics and socio- economic status are possible reasons for the different prevalence of ASB [15]. The women who participated in this study were popularly of low socioeconomic status considering that majority were unemployed and earning nothing every month, a possible explanation of the high prevalence of 14.2% [15]. The settings for this study are located in the capital of Zimbabwe, one of the developing nations, where higher ASB prevalence than the general is not surprising. A higher prevalence than obtained in this study could have been obtained in this study if the women included were in the second and third trimester as gestational age is another factor for ASB.

The Griess test is very effective in identification of all bacterial uropathogens which have an enzyme which is responsible for conversion of nitrate normally found in urine to nitrite [15]. This characteristic is typical of all gram negative bacteria some of the gram positive bacteria where presence of nitrite signifies presence of a urinary tract infection [15,16]. Culture test of midstream urine is the gold standard test for diagnosis of ASB in pregnancy [16]. The significance level for diagnosis of ASB is not standard although currently 10^5 cfu/ml is being used. This value is the same being used for acute urinary tract infection. However some argue that bacterial colony from 10^3 cfu/ml could be an indication of an early phase of the infection but a less value should be considered a contamination [14]. In this study a significance level of 10^3 cfu/ml was used to confirm diagnosis of ASB.

Gram negative bacteria are generally responsible for the highest cases of ASB causing 70% to 90% or more ASB cases [11]. Meanwhile gram positive bacteria also play a role in occurrence of ASB. A wide range of these bacteria is responsible for ASB, with *E. coli* being carrying the greatest responsibility of the cases [17]. However, the etiological agents for ASB, as with the other types of UTIs differ from setting to setting [17].

In this study *CoNS* also known as *Staphylococcus saprophyticus,* was responsible for the highest (29.4%) ASB cases followed by *E. coli* (23.5%). *CoNS* was historically considered a contaminant before the 1960s, being a normal flora of the skin but is now responsible for significant infection even of the urinary tract [18]. *CoNS* was, since 1962, reported to be one of the common agents responsible for UTIs including ASB in pregnancy, with a current responsibility of up to 15% [19]. The bacteria may therefore reside in the urinary tract of the sexually active females [19]. The bacteria is said to be second to *E. coli* as the most common cause of uncomplicated UTI in women [19]. From a study conducted in Dhaka *CoNS* came second (7.01%) after *E. coli* (82.61%) [19]. Results in this study were similar to those found in a study conducted in Ethiopia, where *CoNS* was the most popular (32.6%) bacterial isolate responsible for ASB followed by *E. coli* (26.1%) 9) [20]. In separate studies conducted in Kenya and India, *E. coli* was the popular uropathogen [6,21].

The other popularly identified bacteria causing ASB in pregnancy include *Staphylococcus aureus* and *Klebsiella pneumonia* [17]. In this study the other uropathogens identified were

Staphylococcus aureus (11.8%), *Klebsiella pneumoniae* (8.8%), *Bacillus* species (8.8%), *Providencia* (2.9%), *Salmonella* (2.9%), Streptococcus viridans (2.9%) and *Shigella* species (2.9%). *Staphylococcus aureus* was also identified in other studies [9,20,22]. *Klebsiella* species are among the commonly identified gram negative bacteria causing ASB, often the second common after *E. coli* [17]. In a study conducted in Nigeria, *Klebsiella* species were the most commonly identified (35.38%) uropathogen [10]. In Kenya *Klebsiella* species were the second most common uropathogen after *CoNS* [6]. Number of cases caused by *Klebsiella* species are even reported to be growing [17].

Bacillus species may either be gram positive or negative and are responsible for UTI among other infections [23]. Meanwhile, they also have ability to reduce nitrate to nitrite [23]. A single case of ASB caused by *Providencia* (1, 6.25%) was identified in a study in Ghana, the same as found in this study. *Shigella* species are commonly known to cause gastrointestinal tract infections but the bacteria are also responsible though rarely, for some urinary tract infections [24]. *Salmonella* species responsible for UTIs are associated with immunodeficiency and structural deformity of the urinary tract [25]. *Salmonella* urinary tract infection is rare but occurs and is associated with haematogenous transfer or directly to the bladder via the urethra [26]. In a study conducted in a prevalence of 15% of *Salmonella* UTI was found [26].

5. CONCLUSION

ASB is caused by a variety of bacteria including both gram negative and gram positive strains. The etiological agents differ from setting to setting. In this study *CoNS* (29.4%) was the most common followed by *E. coli* (23.5%), *Staphylococcus aureus* (11.8%) and *Klebsiella* (8.8%). Knowledge of ASB etiological agents in pregnancy is required to guide prevention and treatment strategies to be able to prevent adverse pregnancy outcomes the infection is associated with.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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