

# A Preliminary Assessment of Asymptomatic Bacteriuria of Pregnancy in Brunei Darussalam

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## Abstract

**Background:** Asymptomatic bacteriuria describes a condition in which urine culture reveals a significant growth of pathogenic bacteria, specifically greater than  $10^5$  of colony-forming units per millilitre of urine. It has a direct bearing on the health of a pregnant woman, her pregnancy and consequently the foetus. Thus, this study investigated the prevalence of asymptomatic bacteriuria of pregnancy in Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital, the premiere tertiary hospital in Brunei Darussalam.

**Methods:** A total of 170 pregnant women who were visiting the Department of Obstetrics and Gynaecology at RIPAS Hospital for routine antenatal care between February and March 2011 volunteered for this cross-sectional study. They did not present with any clinical symptoms of bacteriuria or indeed any other illness. They were investigated for bacteriuria by urine microscopy, culture, and sensitivity.

**Results:** Urine samples from seven of the women produced significant bacterial growth, showing a prevalence of 4.1%. The organisms isolated were *Klebsiella* species (2.94%) and *Escherichia coli* (1.18%); these bacteria were both sensitive to amoxicillin, vancomycin, tetracycline, and erythromycin.

**Conclusion:** Brunei has a similar prevalence of asymptomatic bacteriuria to other South-East Asian countries.

**Keywords:** asymptomatic, bacteriuria, infections, pregnancy complications, urinary tract infections

## Introduction

Asymptomatic bacteriuria is the term used to describe the colonisation of pathogenic bacteria in clean-catch urine in the absence of clinical symptoms (1). It occurs in both pregnant and non-pregnant women (2), and indeed in both genders at various ages and conditions. Symptomatic and asymptomatic bacteriuria is common in pregnant women (3), and although pregnancy does not increase the prevalence of asymptomatic bacteriuria, it does enhance the progression rate from asymptomatic to symptomatic disease (4). The physiological changes that accompany pregnancy include a weakening of the immune system (5), decreased urine concentration resulting from increased plasma volume and the development of glycosuria in about 70% of pregnant women; these elements encourage bacteria multiplication in urine (6,7). Factors that are associated with increased incidence

of asymptomatic bacteriuria include poor socioeconomic status, age, duration of pregnancy, and multiparity (8). Akinloye et al. (2006), reported that advanced maternal age might be a risk factor for asymptomatic bacteriuria in pregnancy (1). However, women that are 35 years of age or older are likely to have many children before the present pregnancy, and it has been reported that multiparity itself contributes to the risk of asymptomatic bacteriuria (8). Poor genital hygiene, whereby pregnant women find it difficult to clean their anus properly after defecating or after passing urine, is another risk factor which might promote bacterial infection and/or increased chances of bacterial colonisation and may contribute to asymptomatic bacteriuria (9).

Complications for both the mother and the unborn child may arise if asymptomatic bacteriuria is undetected or left untreated (10). These complications can cause pre-term labour and maternal septicaemia risks for symptomatic

urinary tract infection (8), premature delivery, intrauterine growth retardation, and post-partum endometritis (11). Others possibilities are advanced cervical dilatation, pre-eclampsia, anaemia, and chorioamnionitis (1).

The prevalence of asymptomatic bacteriuria in pregnancy varies widely within and between countries. For example, in Iran, the prevalence of asymptomatic bacteriuria in pregnancy has been reported 2–10% (12,13), while it is estimated at 6.2% in India (14), 12% in Bangladesh (15), 7.3% in Ghana (16); 6% in Singapore (17) 1.9% (18) and 4.3% (19) in Malaysia; and 14.6–86% in Nigeria (1,9,20–22). This is in stark contrast to Saudi Arabia, where the prevalence is 1.7% (23). This suggests that several local cultural practices may significantly contribute to the prevalence of this condition; and has thus made it imperative to determine the prevalence of asymptomatic bacteriuria in Brunei Darussalam which, unlike several other countries, has yet to be studied in relation to this topic.

The implications for undetected and/or untreated asymptomatic bacteriuria have prompted some workers in the field to advocate routine urine microscopy, culture, and sensitivity (*mcs*) investigation for all pregnant women (9,24). In RIPAS Hospital, as well as all the antenatal clinics in Brunei, routine urine *mcs* screening had hitherto not being carried out on antenatal patients; rather, clinicians opted for the use of a strip and urinalysis machine to assess urine in pregnant women. While these methods can easily detect glucose and protein and possibly indicate the presence of some bacteria in urine, they are neither able to identify nor quantify the organisms that may be present. They are therefore inadequate for detecting and assessing the extent of infection. Furthermore, the strip urinalysis machine test system is at this time unable to provide the antimicrobial sensitivity patterns needed for effective therapy; such information is usually obtained following urine culture testing.

Due to these considerations, the present study was aimed at determining the prevalence of asymptomatic bacteriuria in patients attending the antenatal clinic of RIPAS Hospital, identifying the organisms involved and determining their antimicrobial sensitivity profiles using the *mcs* testing system.

## Materials and Methods

### Study population

Initial volunteers for this study consisted of 171 pregnant women in the second or third

trimester of pregnancy. They were attending the antenatal clinic at the Department of Obstetrics and Gynaecology, RIPAS Hospital, Brunei Darussalam, where they were recruited. Ethical committee approval for this study was obtained from Brunei Darussalam's Ministry of Health Research and Ethics Committee (MHREC), which is statutorily responsible for ensuring that research conducted on human subjects in the country are in accordance with the humane and ethical principles of research outlined in the Helsinki guidelines. Thus, this study was conducted in strict compliance with the principles of the Helsinki declaration. Briefly, to recruit the participants, all the patients attending the clinic during the months of February and March 2011 were informed of the study. The aims, objectives and study procedure were explained to them and all their questions and concerns were satisfactorily addressed. They were informed of their right not to take part in the study if they so desired without any consequences. Similarly, those who volunteered were informed of their right and freedom to withdraw from the study before the results are reported without any repercussions. They were also informed that the data would be handled with the strictest confidentiality. Those who agreed to take part in the study gave written and signed consent. One of the volunteers withdrew at this stage, such that 170 participants remained.

### Specimen collection

Each participant produced approximately 20 mL of clean voided midstream urine into a sterile, screw-cap universal bottle following appropriate instructions to ensure the samples were free of contamination. The collection of all specimens was directly supervised by an individual nurse throughout the study. Urine samples were transported to the Pengiran Anak Puteri Rashidah Sa'adatul Bolkiah (PAP RSB) Institute of Health Sciences Research Laboratory within two hours in a container filled with ice. Extra care was taken to maintain the sterility of the containers by ensuring that there was no spillage from any of the universal bottles. The specimens were immediately processed or if processing was not immediately possible, refrigerated at 4 °C; processing was carried out within four hours of specimen collection.

### Specimen processing

Using a sterile centrifuge tube, approximately 10 mL of each well-mixed urine sample was centrifuged at 3000 rpm for 10 minutes. After

discarding the supernatant, a drop of a properly mixed deposit was microscopically examined at 10× and 40× magnifications to detect the presence of pus cells, red blood cells, epithelial cells, casts, crystals, yeast-like cells, and *Trichomonas vaginalis*.

Employing a sterile, disposable calibrated loop delivering 0.002 mL of urine, a loopful of the sediment of the centrifuged urine specimen was streaked using the four-way streak method on blood agar (BBLTM Blood Agar Base Infusion Agar with 5% of sterile, defibrinated blood) and MacConkey agar to produce discrete colonies. New sterile calibrated loops were used for each streak made. The plates were incubated aerobically at 36.5 °C for 24 hours. The inoculation of the specimen was done aseptically to prevent any possibility of false-positive results due to contamination while carrying out the procedure. The supernatant of the centrifuged urine was tested with Bayer (Siemens) Multistix 10SG strips. The presence of nitrite and leukocyte esterase in the urine was taken as being suggestive of an infection, as well as the presence of pus cells greater than 5 per high field (PHF).

Growths on the blood agar and MacConkey agar plates were observed after 24 hours of aerobic incubation at 36.5 °C. Colony-forming units (cfu) were estimated and bacterial isolates with growth of more than 10<sup>5</sup> cfu/mL of urine were regarded as representing significant bacteriuria. The organisms were identified following standard procedures (25).

Bacterial isolates with significant growth were subjected to antibiotic sensitivity testing

following the National Committee for Clinical Laboratory Standards for Antibacterial Disc Susceptibility Tests (NCCLS) recommendations (26). The antibiotic discs used were amoxicillin, vancomycin, tetracycline, clavulanic acid, sulphamethoxazole, erythromycin, and penicillin G.

## Results

Out of the 170 urine specimens tested from the volunteering pregnant women, 7 samples showed significant growth on culture, giving a prevalence of 4.1% of asymptomatic bacteriuria, while 163 (95.9%) showed no significant growth or no growth at all. Bacteriuria involves a significant growth of bacteria with 10<sup>5</sup> or more cfu/mL of urine (1,23). Five of the urine samples grew *Klebsiella* species with a frequency of 2.94%, and the remaining two grew *E. coli* with frequency of 1.18%. Four of the seven women with significant bacterial growth in their urine samples were in their late second trimester, while the remaining three were in the early third trimester. The age range of the seven women was between 20 and 35 years. The prevalence of uropathogens is shown in table 1.

Each of the seven urine samples with significant bacterial growth also had more than 5 pus cells PHF. The presence of more than 5 pus cells PHF indicates infection (9). As might be expected, dipstick analysis with Multistix test strips showed positive test results for leukocyte esterase in all seven urine samples with significant bacterial growth. A positive leukocyte

**Table 1:** Prevalence of pathogenic bacteria in urine samples of pregnant women (n = 170)

Organism	Number (%) prevalence
Normal (no organisms detected)	163 (95.88%)
Total number of infected samples	7 (4.12%)
<i>Klebsiella</i> species	5 (2.94%)
<i>Escherichia coli</i>	2 (1.18%)

**Table 2:** Antibiotic sensitivity results of bacterial isolates

Bacteria	No. (%) sensitive				
	No. tested	AMX	ERY	VAN	TET
<i>Klebsiella</i> species	5	5 (100%)	4 (80%)	5 (100%)	3 (60%)
<i>Escherichia coli</i>	2	2 (100%)	2 (100%)	2 (100%)	1 (50%)

Abbreviations: AMX = amoxicillin; ERY = erythromycin; VAN = vancomycin; TET = tetracycline.

esterase test is indirect evidence for the presence of bacteriuria (11). Both the isolated *E. coli* and *Klebsiella* species were sensitive to amoxicillin, vancomycin, tetracycline, and erythromycin as the choices of antibiotics for both species. The antibiotic sensitivity results of the isolates are shown in table 2.

## Discussion

The prevalence of asymptomatic bacteriuria in pregnancy in RIPAS Hospital, Brunei Darussalam was 4.1% in this study. The implication of this is that about 4.1% of the pregnant women participants in the study are at risk of developing acute episodes of urinary tract infections and related complications if they are not treated. This may result in various complications for both the mother and foetus, which may include pre-term labour and maternal septicaemia (8), pre-mature delivery, intrauterine growth retardation and post-partum endometritis (11), advanced cervical dilatation, pre-eclampsia, anaemia and/or chorioamnionitis (1).

At 4.1%, the prevalence of asymptomatic bacteriuria is similar to that reported in a study carried out at the Hospital Universiti Sains Malaysia in 2003, which was 4.3% (17); on the other hand, it is much higher than the rate of 1.9%, which was obtained in a study carried out in the same country by Mohammad et al. (2002) using a different population (19). In the Kandang Kerbau Maternity Hospital in Singapore, the prevalence of asymptomatic bacteriuria in a similar study in 1968 was found to be 6% (17), while in India, it was 6.2% (14). In contrast, several other countries have shown very much higher prevalences of asymptomatic bacteriuria. For example, the prevalence in Iran ranges between 2% and 10% (12, 13); in Ethiopia, it is 7% (27); Ghana, 7.3% (16); Bangladesh, 12% (15); and in Nigeria it is in the range of 21–86% (1,9,20–22). All of these rates are higher than the 1.7% of pregnant women identified in Saudi Arabia (23) and 1.9% in the Philippines (28).

The wide variations in the prevalence of asymptomatic bacteriuria within and between countries and regions are very striking and may reflect variations related to population characteristics such as age, parity, socioeconomic status, sexual activity and health care during pregnancy (23,29). They may also be due to setting of the study (primary care, community based, or hospitals) or the variation in the screening tests employed (urine dipstick, microscopy, and culture) (23), as well as whether

the studies were prospective or retrospective. Furthermore, while race-specific variation may exist, it is likely to be superseded by geographical and/or socioeconomic factors. For example, the prevalence of asymptomatic bacteriuria amongst pregnant women of Bangladeshi origin living in London is 2% (30), whereas in women living in rural Bangladesh, it is 12% (15). Thus, with regards to health planning, antenatal care, and treatment strategies, it is imperative to determine the prevalence of asymptomatic bacteriuria in every community or geographical population given the risks of undetected and/or untreated cases to maternal and neonatal health.

The organisms found in this study were *Klebsiella* species (71.4%) and *Escherichia coli* (29.6%). Other studies have reported the presence of group B *Streptococcus*, diphtheroids and *Candida albicans* (23). This differs from the findings from the Hospital Universiti Kebangsaan Malaysia, where *E. coli* was the most common bacterium, with a frequency of 40%, followed by group B *Streptococcus* (15%), *Klebsiella* species (15%), diphtheroids, and *Candida albicans*. One possible reason for the lower number of bacterial types in our study may be the relatively smaller number of study volunteers in comparison to the studies from other countries mentioned above. It is pertinent to say, however, that our study population is also a great deal smaller than that for any of the countries reported, as Brunei is a much smaller country whose population that is less than 0.5 million people, including expatriates (31). Indeed, as a proportion of the countrywide population, the sample size in our study compares favourably with those of the previous research. It is highly likely that with a longer period of time and a wider coverage to extend our future study, more diverse microorganisms could be found with similar frequencies to those reported in previous research.

The standard guideline followed globally to confirm asymptomatic bacteriuria is when two consecutive urine specimens show significant growth in the culture (32). In this study, only one sample collection was used. It was not possible to test a second urine specimen from the same patient to confirm asymptomatic bacteriuria due to the inability to track patients. This is because the recording of personal unique identification of patients who participated was not permitted by the Research and Ethics Committee during the study, and the results were kept anonymous. The only information that could be recorded was age and period of pregnancy. However, other workers have determined the prevalence of asymptomatic



bacteriuria in pregnancy based on one sample, with no follow-up (1,9,16; and 3 for review). Thus, our experimental protocol for this study is an acceptable practice in the field.

Most of the patients visiting the Department of Obstetrics and Gynaecology in RIPAS Hospital were in their second or third trimester. None of the patients that volunteered for this study was in their first trimester; therefore, the study lacks information related to comparing the prevalence and presence of asymptomatic bacteriuria within all three trimesters of pregnancy. Early detection of asymptomatic bacteriuria is essential to prevent complications that may arise later in pregnancy. We therefore propose to extend our future study to include pregnant women in their first semester.

## Conclusion

We investigated the presence of bacteriuria in antenatal patients attending RIPAS Hospital, Brunei Darussalam by urine microscopy, culture, and sensitivity. Urine samples from seven of the women produced significant bacteria growth, exhibiting a prevalence of 4.1%. The isolated *Klebsiella* species (2.94%) and *Escherichia coli* (1.18%) were both sensitive to amoxicillin, vancomycin, tetracycline and erythromycin. There were no mixed infections and the prevalence of asymptomatic bacteriuria in this study is comparable to that of the South East Asian region. Given the clinical significance of undetected and/or untreated asymptomatic bacteriuria on health and the disease burden, we intend to follow up this study to cover the four administrative districts in the country. It is our opinion that this will provide data for clinical management of diseases, as well as prudent national health planning.

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## Conflict of Interest

None.

## Funds

None.

## Authors' contributions

Conception and design, analysis and interpretation of the data, final approval of the article : SHM, OA  
Drafting of the article: SNBG, SHM  
Critical revision of the article for the important intellectual content: OA  
Provision of study materials or patient: RY  
Statistical expertise, obtaining of funding: SNBG, SHM, OA  
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