ASYMPTOMATIC BACTERIURIA IN EGYPTIAN PREGNANT FEMALES

M.Sc. Thesis Submitted for the Fulfillment of M.Sc. Degree in Medical Microbiology and Immunology

By

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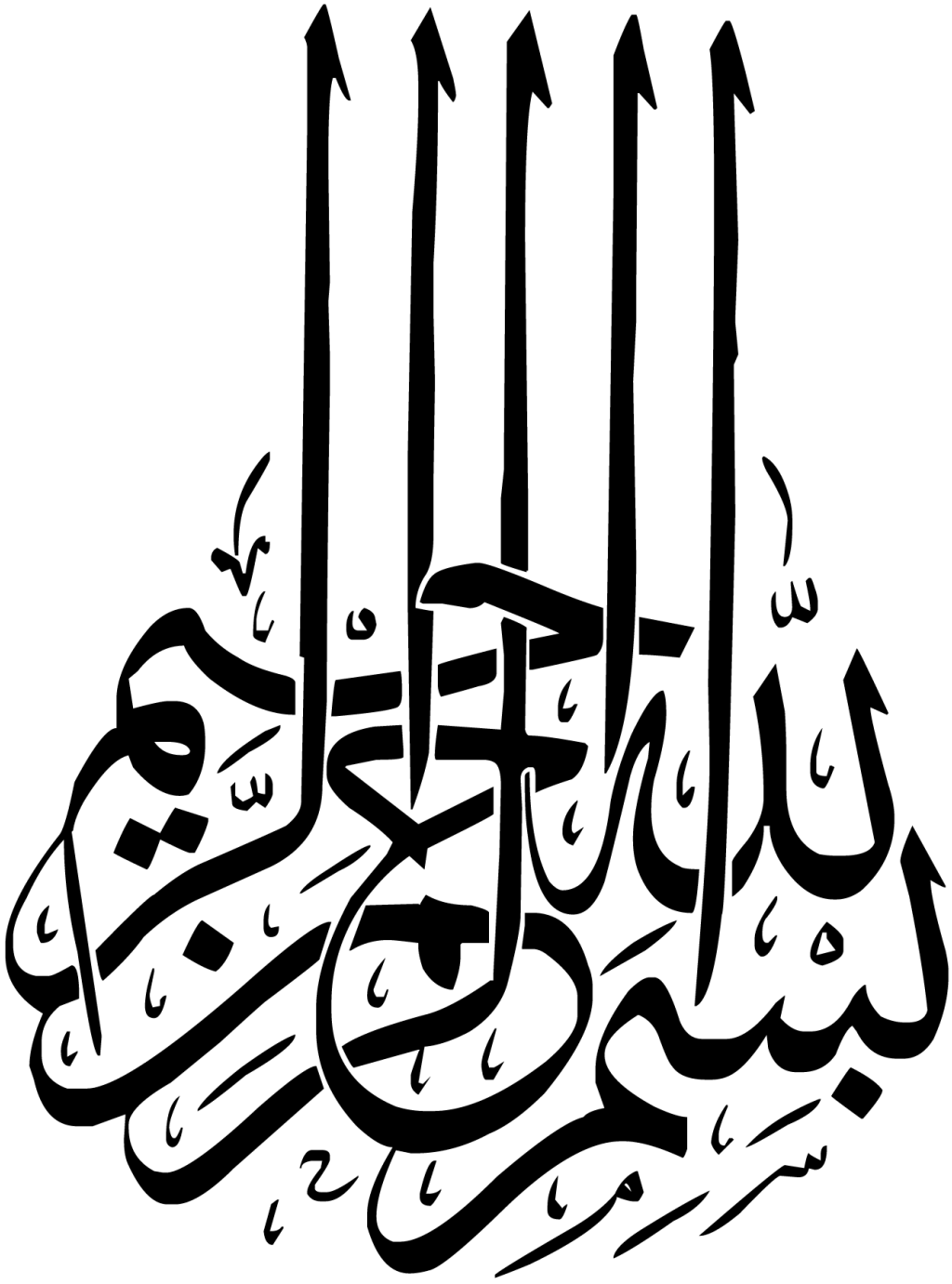
بسم الله الرحمن الرحيم

CONTENTS

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LIST OF TABLES

LIST OF FIGURES

Abstract

Asymptomatic bacteriuria (ASB) describes a condition in which urine culture reveals a significant growth of pathogenic bacteria, specifically greater than 10^5 of colony-forming units (CFU) per millilitre of urine. It has a direct effect on the health of a pregnant woman, her pregnancy and consequently the fetus.

The aim of this study was to determine the prevalence of ASB in pregnant women and also to isolate, identify and establish antimicrobial susceptibility pattern of the pathogens responsible for ASB, to assess possible associated risk factors. Follow up of ASB positive cases to assess maternal and fetal complications was also done.

Urine samples were collected from 256 asymptomatic pregnant women. Microscopic examination of centrifuged samples was done. Urine culture is considered the gold standard method for diagnosis of ASB during pregnancy, cultivation on CLED by standard loop technique was done to the samples.

Our results showed that ASB prevalence rate was 7.8% among the studied group. *E. coli* was the most common uropathogen isolated (35%), followed by *Enterococci* (25%), *Klebsiella* (15%) one *K. oxytoca* and two *K. pneumonae*, *S. aureus* (10%), *Acinetobacter* (10%) and *CoNS* (5%).

The in vitro susceptibility pattern of 20 isolates to different antibiotics was measured by disc diffusion method. This study revealed that nitrofurantoin (90% sensitivity), imipenem (80%), norfloxacin (75%) and amikacin (75%) were very effective against most of the urinary isolates. Co-trimoxazole (60%), augmentin (55%), ceftazidime (50%) and ceftriaxone (45%) were moderately effective against the urinary isolates, while most of the urinary isolates were resistant to cephalexin, cefuroxime and cefotaxime.

Keywords: Asymptomatic bacteriuria, Pregnancy, Urine culture, Antibiotic sensitivity.

Introduction

Asymptomatic bacteriuria (ASB) is a common health problem in women and increases in prevalence with age and/or sexual activity, due to short urethra, pregnancy, easy contamination of urinary tract (UT) with fecal flora **(Enayat *et al.*, 2008; Girishbabu *et al.*, 2011)**.

ASB is found in 2-10% of pregnant women **(Enayat *et al.*, 2008)**. Detection of ASB in antenatal women is important, as, undetected and untreated ASB may lead to symptomatic infection during pregnancy in 25% of culture positive patients, acute pyelonephritis, hypertension in pregnant women, postpartum urinary tract infection (UTI), anemia, preterm labour, low birth weight and perinatal death of the fetus **(Cunningham *et al.*, 2001; Enayat *et al.*, 2008)**.

ASB is defined as the presence of at least 10^5 colony-forming units (CFU)/ml of one or two bacterial species in clean-voided midstream urine sample from an individual without symptoms of UTI **(Harding *et al.*, 2003)**.

The pregnant women are two times more commonly affected than age matched non-pregnant females. This is due to urinary stasis due to progesterone effect in pregnancy in addition to different morphological and physiological changes occurring during pregnancy **(Chandel *et al.*, 2012)**.

The prevalence of ASB is about 3 times higher in diabetic women, ranging from 15-30%, than in non-diabetic women; less than 10% (**Harding *et al.*, 2003)**.

Urine culture is the gold standard screening technique for ASB during pregnancy **(Gayathree *et al.*, 2010)**. Gram- negative bacteria were mainly responsible for ASB. The most common infecting organism is *Escherichia coli*, which is responsible for 75-90% of bacteriuria during pregnancy, followed by *Klebsiella* spp., *Enterobater*, and *Pseudomonas aeruginosa* **(Papazafiropoulou *et al.*, 2010)**.

Gram-positive organisms have recently received more attention as causing bacteriuria and UTI. Although, they are seen in small numbers during pregnancy, they are recognized as important causes of UTI **(Fareid, 2012)**.

Early detection and treatment is of considerable importance not only to prevent acute pyelonephritis and chronic renal failure in the mother, but also to reduce prematurity and fetal mortality **(Gayathree *et al.*, 2010; Girishbabu *et al.*, 2011)**.

Antibiotic-resistant organisms that cause community acquired UTI include gram-negative organisms particularly those species that produce *Amp C* enzymes or extended-spectrum β-lactamases (ESBLs). Urea-splitting organisms such as *Proteus* spp., *Morganella morganii* and *Providencia* are often found in patients with indwelling devices. Gram-positive cocci such as methicillin-resistant *S. aureus*, methicillin-resistant coagulase-negative staphylococci and vancomycin-resistant enterococci are also problematic **(Nicolle, 2005)**.

AIM OF THE WORK

The present study is conducted to:

1) Determine prevalence of ASB in pregnant females.
2) Follow up pregnant women with ASB for determining the frequency of complications and possible risk factors in them.
3) Determine aerobic bacteria causing ASB in pregnant females.
4) Determine antibacterial susceptibility pattern of isolated organisms.

ASYMPTOMATIC BACTERIURIA IN PRGNANCY

ASB is defined as the presence of actively multiplying bacteria of more than or equal to 10^5 CFU per ml in clean-voided midstream urine sample from an individual without symptoms of acute UTI **(Greval *et al.*, 2003)**.

According to **Nicolle *et al.*, 2005,** ASB is defined as:

1. A single, clean-catch voided urine specimen with one bacterial species isolated in a quantitative count $\geq 10^5$ CFU/ml identifies bacteriuria in men.
2. For asymptomatic women, bacteriuria is defined as two consecutive voided urine specimens with isolation of the same bacterial strain in quantitative counts $\geq 10^5$ CFU/ml.
3. A single catheterized urine specimen with one bacterial species isolated in a quantitative count $\geq$ 100 CFU/ml identifies bacteriuria in women or men.

If there are more than 10^5 CFU/ml in a clean-catch urine, there is 80% probability that it is true bacteriuria. If two different samples demonstrate the same specimen at least $\geq 10^5$ CFU/ml, the probability increases to 95% **(Sobel and Kaye, 2006)**. However, after 7-10 days, the second culture will be positive only in about 65%. This observation supports the possibility of spontaneous resolution of ASB (**Dalal *et al.*, 2010)**.

Pregnancy enhances the progression from ASB to symptomatic bacteriuria (SB). ASB needs special attention, due to lack of symptoms and its adverse consequences in pregnancy, which could lead to pyelonephritis and adverse obstetric outcomes **(Kerure *et al.*, 2013)**.

Causative Organisms

E. coli is the most common organism isolated from patients with ASB. However, the infecting organisms are diverse, within *Enterobacteriaceae; Proteus, Klebsiella, Enterobacter, Citrobacter* species, *Pseudomonas aeruginosa,* gram-positive organisms, as staphylococci, group B streptococcus (GBS), *Enterococcus* species and others; *Gardnerella vaginalis and Ureaplasma urealyticum* **(Macejko and Schaeffer, 2007)**.

Prevalence

ASB is common, but the prevalence in populations varies widely with age, sex, and the presence of genitourinary abnormalities. For healthy women, the prevalence of bacteriuria increases with advancing age **(Nicolle *et al.*, 2005)**. The prevalence of ASB is 3 – 12% among pregnant mothers, as revealed by different studies **(Ullah *et al.*, 2007; Assefa *et al.*, 2008; Hooton, 2010; Celen *et al.*, 2011; Ipe *et al.*, 2013)**.

Risk Factors

Whether bacteriuria becomes symptomatic UTI or ASB is determined by interplay of host response to the pathogen and the virulence of the organism **(Trautner, 2012; Nicolle, 2014)**.

- **Host factors:**

They include genetic predisposition, sex (female, pregnancy, sexual activity, and method of contraception), age and parity, diabetes mellitus, anemia, and socioeconomic status.

1) Genetic predisposition:

Raz *et al.*, (2000) showed that there is genetic predisposition to SB. The innate immune response of the UT to bacterial invasion involves toll-like receptors (TLRs) that recognize pathogens and chemokine receptors that trigger neutrophil recruitment **(Ragnarsdottir *et al.*, 2008)**.

Hernandez *et al.*, 2011 suggested that the host-specific immune response to ASB is mainly determined through innate immune mediators. The host immune response is less vigorous with ASB than with symptomatic infection. Polymorphism of receptors involved in the inflammation process seems to be involved in the degree of susceptibility to developing symptomatic UTI **(Minardi *et al.*, 2011)**.

After binding to uroepithelial cell receptors and expression of virulence factors by uropathogens, TLRs are activated. Subsequently, they activate the innate immune response, along with production of cytokines **(Fischer *et al.*, 2006)**. Cytokines are essential to modulate the immune response. IL-8 is involved in neutrophil recruitment and activation by binding to the CXCR1 receptor **(Wullt *et al.*, 2010)**.

TLR4 is a receptor expressed on neutrophils and shown to play an important role in the host response to uropathogens. TLR4 is the host immunity factor with the best-defined relationship to ASB; reduced TLR4 expression and signaling are both associated with ASB in children **(Fischer *et al.*, 2006; Ragnarsdottir *et al.*, 2007; Nielubowicz and Mobley, 2010)**. Certain polymorphisms in the TLR4 promoter can lead to an attenuated immune response, promoting the asymptomatic carrier state **(Ragnarsdottir *et al.*, 2010)**.

After binding of uropathogens to the receptor, the main cytokine involved in the response is IL-8, which binds to the CXCR1 receptor on the

neutrophil plasma membrane. CXCR1 mediates the migration of uropathogens through the urothelial wall, leading to pyuria. IL-8 levels in the blood have been demonstrated to correlate positively with the number of neutrophils found in the urine during infection **(Minardi *et al.*, 2011)**.

Absence of receptor CXCR1 has recently been shown to promote bacterial growth within the UT **(Hawn *et al.*, 2009)**. Low CXCR1 expression was associated with an inherited susceptibility to pyelonephritis **(Lundstedt *et al.*, 2007)**.

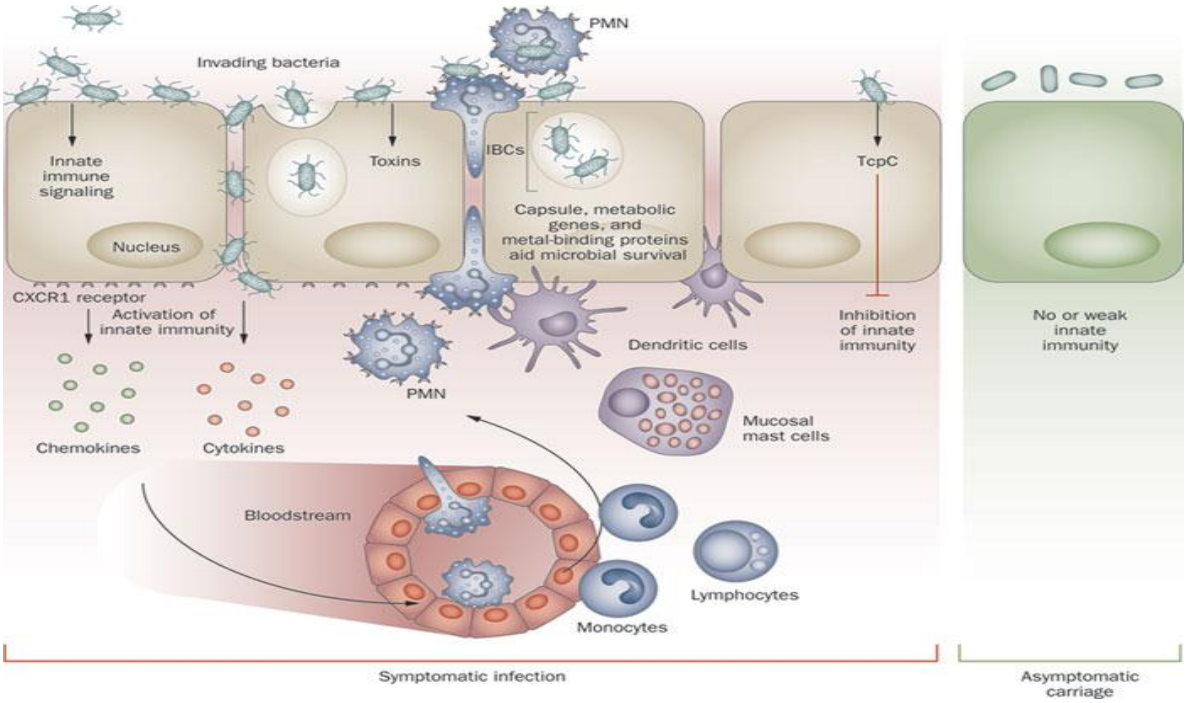

Figure (1): Immune response to symptomatic and asymptomatic UTI (Brendis *et al.*, 2011)

2) Sex:

Women tend to have ASB more often than men because bacteria can reach the bladder more easily in women. This is partially due to the short and wider female urethra and its proximity to anus. Bacteria from the rectum can easily travel up the urethra and cause infections (**Feitosa *et al.*, 2009; Kolawole *et al.*, 2009; Girishbabu *et al.*, 2011**).

The pregnant women are two times more commonly affected than age matched non-pregnant females. This is due to urinary stasis due to progesterone effect in pregnancy in addition to different morphological and physiological changes occurring during pregnancy **(Chandel *et al*., 2012)**.

Pregnancy is a unique state with anatomic and physiologic UT changes. The renal pelvis and ureters begin to dilate as early as the eighth week of pregnancy and the bladder itself is displaced superiorly and anteriorly **(Jeyabalan and Lain, 2007; Ansari and Rajkumari, 2011)**. Mechanical compression from the enlarging uterus is the principle cause of hydroureter and hydronephrosis, but smooth muscle relaxation induced by progesterone results in decreased peristalsis of the ureters, increased bladder capacity and urinary stasis **(Schnarr and Smaill, 2008; Perera, 2009)**.

Decreased urine concentration resulting from increased plasma volume, differences in urine pH and osmolarity and pregnancy-induced glycosuria and aminoaciduria may facilitate bacterial growth **(Jeyabalan and Lain, 2007; Ansari and Rajkumari, 2011)**.

ASB is common in sexually active females as the pathogen entry is facilitated by sexual activity **(Feitosa *et al*., 2009; Girishbabu *et al*., 2011)**. **Scholes *et al*., (2000)** found that women who have been sexually active within the past month are six-times more likely to present with infection compared with women who are not sexually active.

Gupta *et al*., (2000) showed that women who use spermicides for birth control have an increased vaginal pH and increased colonization with potential uropathogens, particularly *E. coli.*

3) Age and parity:

Girishbabu *et al*., (2011) found that prevalence of ASB increases with higher parity and advancing age. There is a strong association between