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Research Article

Prevalence of Multi Drug Resistance Asymptomatic Bacteriuria from Pregnant Women in Karaikudi, Sivagangai District

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Abstract

Asymptomatic bacteriurias (ASB) during pregnancy are among the commonest health problems worldwide, especially in developing countries, including India. In this view, the present study was undertaken to determine the prevalence of multidrug resistant asymptomatic bacteriuria from pregnant women in the Sivagangai district. A total of 76 were positive for significant bacteriuria. It was noted that asymptomatic bacteriuria were high in pregnant women. Out of 76 positive samples, highest prevalence of bacteriuria was observed in pregnant women within the 20 and >30 years age group were between 32 (30.76%) and 21 (30.88%). Microscopic field contained more than five leukocytes (WBCs) or erythrocytes (RBCs) were taken as positive. Microscopic examination of urine samples revealed that 63 (82.89%) were have leukocytes, while 41 (53.94%), 52 (68.42%), 35 (46.05%) and 06 (7.89 %) were have erythrocytes, epithelial cells, crystal and yeast respectively Effect of pathogenic bacteria on ASB was studied and it was found that Escherichia coli cause the highest percentage of infection (31.57%) in pregnant women. The sensitivity rates of isolates were Ciprofloxacin 88.15 % (64), Chloramphenicol 88.15% (64), Gentamicin 77.63% (59) and cefotaxime 72.36% (55). They were found to be the most effective antibiotics against the asymptomatic bacteriuria isolated.

Keywords: Asymptomatic bacteriurias; Pregnancy women; Urinary tract infection; Infection.

Introduction

Urinary tract infection (UTI) is one of the most common diseases in all age groups of male and female, but it is more prevalent in women than in men [1]. Healthy women were more susceptible to severe infectious complications, arising from conditions such as asymptomatic and symptomatic urinary tract infections, particularly pregnant women are relatively common problems during pregnancy ensues, the foetus is still at risk for prematurity, low birth weight and even fetal wastage [2]. Asymptomatic bacteriuria (ASB) is defined as the presence of significant growth of pathogens that is greater than 10^5 bacteria/millilitres in clean-voided midstream urine sample from an individual patient without symptoms of a UTI [3].

The global prevalence of ASB pregnant women ranges from 4% to 23.9% in various studies [1,4]. According to the literature, history of recurrent UTI, Low socio-economic status, increasing maternal age, increasing period of gestation, multiparity, anaemia are all contributory risk factors associated with increased prevalence of asymptomatic bacteriuria in pregnancy [5,6]. The high prevalence of untreated asymptomatic bacteriuria during pregnancy progresses to development of pyelonephritis and associated with an increase in foetal and maternal morbidity [7]. However, several studies have shown that the prevalence of ASB in developing countries is higher than in the developed countries.

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A number of investigate reports have shown the overall prevalence of asymptomatic bacteriuria, *Escherichia coli* is the most common organism associated with bacteriuria, representing at least 80- 90% of isolates, with other gram-negative rods and certain gram-positive organisms including *Staphylococcus saprophyticus* and *Staphylococcus aureus* occasionally being isolated [8-11].

Last few decades increasing prevalence of bacterial resistance to antibiotics mostly associated with the extensive use of antimicrobial agents for UTI. Nowhere has this issue been of greater concern than with the gram-negative and gram-positive bacteria. Multidrug resistance is now the norm among these pathogens [12]. There is no doubt that antimicrobial therapy is necessary when urinary tract infection (UTI) develops in pregnancy may also help to reduce adverse outcome for the child such as preterm labour and low birth weight [13].

This study was therefore conducted to investigate the prevalence of asymptomatic bacteriuria in pregnant women at Karaikudi, Sivagangai District to identify the organisms involved, for further studies their relative proportions and their antibiotic sensitivities. The clinical records were analyzed and statistical analysis was performed using chi-square test.

Methodology

Study population

Urine samples were collected from a total number of 298 pregnant women between the ages of <20 to >30 years, during January to December, 2015. All patients were interviewed during the visit of the study and their medical history was obtained using a standardized questionnaire. The questionnaire included age, UTI surgery during the previous years, and pregnancy trimester, parity, and education level, place of living and socioeconomic status.

Urine collection

Clean catch urine samples were collected in sterile universal containers. 320 "clean catch" mid-stream urine (MSU) samples were collected inside sterile disposable universal bottles from a healthy non-pregnant and pregnant woman. They were instructed on how to collect samples and the need for prompt delivery to the laboratory. The samples were labeled and transported to the Medical Microbiology laboratory, Alagappa University, Karaikudi, Tamilnadu, India in iced pack and were analysed within one hour of collection.

Microscopic examination

For microscopic examination of the urine, 10 to 15 ml aliquots were spun at 1,000 rpm for 5 min. A drop of the resulting sediment was transferred to a microscope slide. Examination for pus cells, red blood cells, epithelial cells, crystals and yeast-like cells was done under both low and high magnifications of the light microscope using standard criteria [14].

Sampling and bacteriological analysis

ASB was defined as the presence of at least 10^5 CFU/ml of one or two bacterial species in a culture. The urine was plated onto 5% sheep blood agar (Hi-media, Mumbai) and MacConkey agar (Hi-media, Mumbai) plates. A calibrated loop designed to deliver 0.01 millilitre of urine was used for urine inoculation [15]. The plates were streaked by touching the loop to the centre of the plate, from which the inoculum spread in a line across the diameter of the plate. Then, loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies. Once plated, urine cultures were incubated at 35ºC aerobically. The results were read after 24 hours. Colonies were counted on each plate [16]. The number of CFUs was multiplied by 100 to determine the number of microorganisms per millilitre in the original specimen. Microorganisms were identified according to standard bacteriologic procedures [17].

Sensitivity tests

Antibiotic sensitivity testing was done by emulsifying selected isolates in normal saline at a turbidity compared to 0.5 Mac Farland's standard. Using sterile swabs, suspensions were inoculated on Muller-Hinton agar in accordance with modified Kirby-Bauer method and incubated at 35 to 37°C for 18 to 24 hrs. Single antibiotic impregnated discs were tested against gram-positive and gram-negative organisms as appropriate. Interpretation was done by comparing the diameter of zone of inhibition with those of a standard table in three grades of susceptibility which are sensitive, intermediate and resistant.

Results and discussion

Out of 298 pregnant women 76 were found positive for significant bacteriuria giving a prevalence of 25.5% (*P*- value <0.048) while 222 (74.49 %) had no appreciable bacterial growth. More than 50% of women have at least one UTI during their life time. UTIs are also a common problem in pregnancy due to the increase in sex hormones and physiological changes during pregnancy [18]. Our results indicated that a highly significant (P< 0.05) prevalence of ASB in pregnant women was 22.5%. This was close to average of prevalence found in previous studies having been reported as 22.2% and 23.9% [19] have also been reported in separate studies. The prevalence of asymptomatic bacteriuria was reported to be as low as 5% in a studied from Pakistan [20] India 6.1 % [21] and 9.5% in another studied from Gahan [22].

The relationship of maternal age with ASB was defined. Mean age of study population was found to be 25.33 ± 5.85 SD. Out of 76 positive samples, highest prevalence of bacteriuria being observed in pregnant women within the 20 and >30 years age group were between 32 (30.76%) and 21 (30.88%) (Table 1). Microscopic field contained more than five leukocytes (WBCs) or erythrocytes (RBCs) were taken as positive. Microscopic examination of urine samples revealed that 63 (82.89%) were have leukocytes, while 41 (53.94%), 52 (68.42%), 35 (46.05%) and 06 (7.89 %) were have erythrocytes, epithelial cells, crystal and yeast respectively (Table 2). In analyzing the studied respondents by age, the highest prevalence of asymptomatic bacteriuria was observed in pregnant women within >30 years age group followed by the 20 to 30 years age group and then the <20 years age group.

Table 1. Pregnant - Mean age 25.33, SD - 5.85947

Age	Positive	%	Negative	%	Total	Chi-square	6.039
<20	23	18.5	103	81.74	126	degrees of freedom	2
20- 30	32	30.76	72	69.23	104	*p*-value	0.048
>30	21	30.88	47	69.11	68	Yates' chi-square	5.139
Total	76	25.5	222	74.49	298	Yates' *p*-value	0.076

Table 2. Microscopic examination of urine samples

S. No.	Microscopic examination	No. of positive samples (%)
1	Pus cell (WBC)	63 (82.89)
2	RBC	41 (53.94)
3	Epithelial cell	52 (68.42)
4	Crystals	35 (46.05)
5	Yeast	06 (7.89)

The observed trend of bacteriuria in this studied and reports from other studies shows the age range of 20 above >30 years serving as a risk group for developing UTI in pregnant women. Effect of age on ASB was studied and found that age group >30 years had the highest percentage of infection (30.88%) and is followed by age group 20 to 30 years (30.76 %) and then is closely followed by age group <20 yrs with 18.5% infected population. The same results were observed in previous reported by Shahira in Egypt but this was not consistent with another reported by Samad Hazhir in Iran, in which highest prevalence (9.9%) was recorded in the age group <20 years and lowest rate (4.7%) between 20 to 30 year of age [23]. However, found that advanced or younger maternal age was not a risk factor related to ABU in pregnancy. So, in this studies have demonstrated that increasing age was associated with an increasing in prevalence in prevalence of asymptomatic bacteriuria in pregnant women.

Table 3 shows the risk factors related to asymptomatic bacteriuria. Out of 76 positive asymptomatic pregnant women sampled, 27 (27.47%) were within the first trimester of pregnancy; 14 (15.21%) were in their second trimester of pregnancy and 35 (35.00%) in their third trimester of pregnancy for significant bacteriuria. Regarding parity 12 (12.24%) patients were nulliparous, followed by primigravida and multigravida were 24 (24.74%) and 40 (38.83%). No relationship between education level and ASB was found as 36 (36.58%) women were Primary, 36 (28.12%) educated up to matriculation and 11 (11.36%) were above ranges. The prevalence of bacteria isolated from the 76 positive cases is shown in

Table 4. The prevalence of *Escherichia coli* was 31.57% (24/76) which ranked as the most prevalent isolated organism followed by *Klebsiella pneumonia* 17.10% (13/76), *Staphylococcus saprophyticus* 14.47 (11/76), *Proteus mirabilis*13.15% (10/76) *Staphylococcus aureus* 11.84% (9/76), *Pseudomonas aeruginosa* 9.21% (7/76) and *Enterococcus faecalis* 5.6% (4/76).

Table 3. Socio demographic characteristics

Variables	ASB	+Ve %	ASB	-Ve %	Total	X^2 (p-Value)
Trimester						
First trimester	27	25.47	79	74.52	106	
Second trimester	14	15.21	78	84.78	92	9.87 (0.007)
Third trimester	35	35.00	65	65.00	100	
Gravidity						
First	38	45.78	45	54.21	83	
Second	15	13.76	94	86.23	109	28.46
Third	21	24.41	65	75.58	86	(0.000)
Fourth +	2	10	18	90	20	
Parity						
Zero	12	12.24	86	87.75	98	18.732
One – Two	24	24.74	73	75.25	97	(0.000)
Three – Four	40	38.83	63	61.16	103	
Socioeconomic status						
Low class	53	31.54	115	68.45	168	7.405
Middle class	23	17.69	107	82.30	130	(0.006)
Education level						
Primary	30	36.58	52	63.41	82	15.024
Secondary	36	28.12	92	71.87	128	(0.000)
Tertiary	10	11.36	78	88.63	88	
Personal hygienic						
Unsatisfactory	56	35.89	100	64.10	156	18.616
Satisfactory	20	14.08	122	85.91	142	(0.000)
Pervious UTI						
Yes	56	35.44	102	64.55	158	17.489
No	20	14.28	120	85.71	140	(0.000)
Residence						
Rural	18	13.53	115	86.46	133	18.114
Urban	58	35.15	107	64.84	165	P 0.000
Total	76	25.5	222	74.49	298	

Table 4. Frequency percentage of isolates

Isolates	Total No of positive culture	Percentage	
Escherichia coli	24	31.57	
Klebsiella pneumonia	13	17.10	
Staphylococcus saprophyticus	11	14.47	X^2=21.795
Proteus mirabilis	10	13.15	(P= 0.001)
Staphylococcus aureus	9	11.84	
Pseudomonas aeruginosa	7	9.21	
Enterococcus faecalis	2		

Of all the bacteria isolated, gram positive and gram-negative bacteria were 100% resistant to ampicillin antibiotics. The highest rate of antibiotic resistance was seen in amoxicillin 76.31% (58) and trimethoprim-sulfamethoxazole 77.63% (59). The sensitivity rates of isolates were Ciprofloxacin 88.15 % (64), Chloramphenicol 88.15% (64), Gentamicin 77.63% (59) and cefotaxime 72.36% (55). They were found to be the most effective antibiotics against the asymptomatic bacteriuria isolated. In the present study, varied antibiotic resistance patterns were observed in the drug-resistant isolates, with resistance to ampicillin, amoxicillin and trimethoprim-sulfamethoxazole being predominant. In present study *Escherichia coli, Klebsiella pneumonia, Staphylococcus saprophyticus, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis* as the most prevalent isolate, showed the high resistant to ampicillin (100%) which was in agreement with other works presented the similar findings. The isolates were highly sensitive to ciprofloxacin 88.15 %, Chloramphenicol 88.15%, Gentamicin 77.63% and Co-Trimoxazole 72.36% (Table 5). It showed excellent antimicrobial susceptibility to all the uropathogens found in this study. These results were mostly in concurrence with other studies [24]. The current study showed high level of ampicillin resistance. This study revealed that ciprofloxacin, chloramphenicol, gentamicin and cefotaxime amikacin, ciprofloxacin, cefuroxime and cefotaxime were very effective against urinary isolates.

Table 5. Antibiotic susceptibility pattern of the isolates.

Isolates	Total	Pattern	AMP % (n=)	AMO % (n=)	TSM % (n=)	CIP % (n=)	CHL % (n=)	CEF% (n=)	GEN % (n=)
Escherichia coli	24	S	0 (0)	29.16 (7)	20.83 (5)	87.5 (21)	79.1 (19)	87.5 (21)	91.66 (22)
		R	100 (24)	70.83 (17)	79.16 (19)	12.5 (3)	20.83 (5)	12.5 (3)	8.33 (2)
Klebsiella pneumonia	13	S	0 (0)	30.76 (4)	23.07 (3)	84.61 (11)	76.92 (10)	46.15 (6)	84.61 (11)
		R	100 (13)	69.23 (9)	76.92 (10)	15.38 (2)	23.07 (3)	53.84 (7)	15.38 (2)
Staphylococcus saprophyticus	11	S	0 (0)	18.18 (2)	9.09 (1)	81.81 (9)	72.72 (8)	54.54 (6)	18.18 (2)
		R	100 (11)	81.81 (9)	90.9 (10	18.18 (2)	27.27 (3)	45.45 (5)	81.81 (9)
Proteus mirabilis	10	S	0 (0)	20 (2)	30 (3)	90 (9)	70 (7)	60 (6)	90 (9)
		R	100 (10)	80 (8)	70 (7)	10 (1)	30 (3)	40 (4)	10 (1)
Staphylococcus aureus	9	S	0 (0)	22.22 (2)	22.22 (2)	88.88 (8)	77.77 (7)	100 (9)	100 (9)
		R	100 (9)	77.77 (7)	77.77 (7)	11.11 (1)	22.22 (2)	0 (0)	0 (0)
Pseudomonas aeruginosa	7	S	0 (0)	1.28 (1)	28.57 (2)	71.42 (5)	71.42 (5)	87.71 (6)	71.42 (5)
		R	100 (7)	85.71 (6)	71.42 (5)	28.57 (2)	28.57 (2)	14.28 (1)	28.57 (2)
Enterococcus faecalis	2	S	0 (0)	0 (0	50 (1)	100(1)	100(1)	50 (1)	100(1
		R	100 (2)	100 (2)	50 (1)	0 (0)	0 (0)	50 (1)	0 (0)
Total	76	S	(0) 0	23.68 (18)	21.05 (16)	88.15 (64)	88.15 (64)	72.36 (55)	77.63 (59)
		R	(100) 100	76.31 (58)	77.63 (59)	14.47 (11)	23.68 (18)	27.63 (21)	21.05 (16)

AMP-Ampicillin, AMO-Amoxicillin, TSM- Trimethoprim-Sulfamethoxazole, CIP-Ciprofloxacin, CHL- Chloramphenicol, CEF- Cefotaxime, GEN-Gentamicin, S-Sentivity, R-Resistant, n= No. of isolates.

Conclusions

Results of the present community based studies have indicated that the asymptomatic bacteriuria in pregnancy was a major health problem in Karaikudi city. The predominant organisms were Escherichia *coli, Klebsiella pneumonia*, *Staphylococcus saprophyticus* and *Proteus mirabilis.* Asymptomatic bacteriuria of pregnancy is difficult to diagnose because of lack of symptoms. If undiagnosed it may led to harmful consequences in pregnancy. We found 25.5 % asymptomatic bacteriuria among our tested patients, so it is therefore recommended that routinely screening for asymptomatic bacteriuria by urine culture. To prevent maternal and perinatal morbidity asymptomatic bacteriuria should be diagnosed and treated as per antimicrobial sensitivity pattern of the isolates. Besides this health education on personal hygiene should be imparted in the antenatal clinic.

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Conflicts of Interest

Authors declare no conflict of interest.

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