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Incidence of asymptomatic Bacteriuria in pregnant women with special reference to *Escherichia coli*

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Abstract
Urinary tract infections (UTIs) are caused by presence and growth of microorganisms anywhere in UT, which is an important cause of morbidity and mortality. Most cases of UTIs are caused by *Enterobacteriacea* especially *Escherichia coli* (*E. coli*), *Klebsiella species* and *Enterobacter species*. The study ascertained the incidence of Bacteriuria in pregnant women with special reference to *E. coli*. This prospective longitudinal study was carried out over a period of two years in Nair Hospital, Mumbai, Subjects were 3000 married pregnant women and 300 non-pregnant women. Collection, transportation and Culturing of urine samples was carried out by standard procedures. Identification of the isolate was done on the basis of morphological, cultural characteristics and Rapid Biochemical Identification Kit. *E. coli* strains were identified by motility and KB003 Hi25™ Enterobactriaceae Identification Kit. Antibiotic susceptibility testing of hundred and eleven *E. coli* isolates obtained from urine samples was carried out by Kirby - Bauer disk diffusion method, *E. coli* was the principal pathogen in pregnant and in non-pregnant women, both, Among the *E. coli* isolates, 84% were sensitive to Piperacillin + Tazobactum followed by to Nitrofurantoin (73%). Around 50% of these strains were found to be sensitive to Norfloxacin, Ceftriaxone, Cefotaxime and Amikacin while remaining were found to be resistant to Ampicillin, Augmentin, Nalidixic acid, Cephalexin, and Cefuroxime. In order to prevent development of resistance, antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications in pregnancy.

Keywords: Urinary tract infections, asymptomatic bacteriuria, *E. coli*, antibiotic susceptibility patterns, pregnant women,

1. Introduction
Approximately 50–60% of women report at least one Urinary tract infections (UTIs) in their lifetime, and one in three will have at least one symptomatic UTI necessitating antibiotic treatment by age 24. *Escherichia coli* (*E. coli*) are responsible for more than 80% of all UTIs and causes both asymptomatic Bacteriuria (ASB) and symptomatic UTI [1]. These infections are typically caused by a single bacterial clone and are in effect monocultures. The ability of uropathogenic *E. coli* (UPEC) to cause symptomatic UTI is associated with the expression of a variety of virulence factors, including adhesins (e.g., type 1 and P fimbriae) and toxins (e.g., hemolysin) [2], Normally, the urinary tract is sterile, but bacteria may rise from the perianal region, possibly leading to UTI. Pathogens in the bladder may stay silent or can cause irritative symptoms like urinary frequency and urgency, and 8% of women may have asymptomatic bacteriuria. If bacteria enter the blood stream, they could cause severe complications, including septicaemia, shock and, rarely, death [3].

The term ASB is used when a bacterial count of the same species over 10^5/ml in mid-stream clean catch urine on two occasions is detected without symptoms of UTI. The apparent reduction in immunity of pregnant women appears to encourage the growth of both commensal and non-commensal microorganisms. Global prevalence of ASB varies widely and in pregnancy, it is 1.9-9.5% [4]. UTIs are caused by presence and growth of microorganisms anywhere in UT, is an important cause of morbidity and mortality. Women are more susceptible than men, due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of UT with faecal flora. It is twice more common in pregnant women than age matched non-pregnants [5]

Prevalence of ASB in non-pregnant women increases with age, sexual activity and parity. And also includes low socioeconomic status, sickle cell trait, diabetes mellitus, grand multiparity. Epidemiology of bacteriuria, risk factors and etiologic agents in pregnancy are similar to non-pregnant women. Bacteriuria in pregnancy usually reflects prior colonization rather than acquisition during the pregnancy itself [6] Organisms that cause UTIs during pregnancy are same as those found in non-pregnant patients. Most cases of UTIs are caused by *Enterobacteriacea* especially *Escherichia coli (E. coli), Klebsiella species and Enterobacter species* which account for 90% of all UTIs encountered in pregnancy. The study aims to ascertain incidence of ASB Bacteriuria in pregnant women with special reference to *Escherichia coli,*

2. Material and methods

2.1 **Place of work**

The study was carried out over a period of two years, from January 2003 to December 2004 after taking the permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai at Department of Microbiology in association with the Department of Obstetrics and Gynecology.

2.2 Participants

The study included the patients from Out-door and Indoor patients of Gynecology department which were recruited for bacteriologic evidence of ASB. Patients included were randomly selected 3000 subjects, married pregnant women-study group and 300 non-pregnant women as control group. Those subjects who were showing signs and symptoms of UTI, suffering from diabetes, under antibiotic treatment in the past, treatment with steroids for any infection / indication was excluded from our study. Counseling for enrollment procedure in the study was done. Detailed data from the patients were recorded in a specially formulated structured proforma.

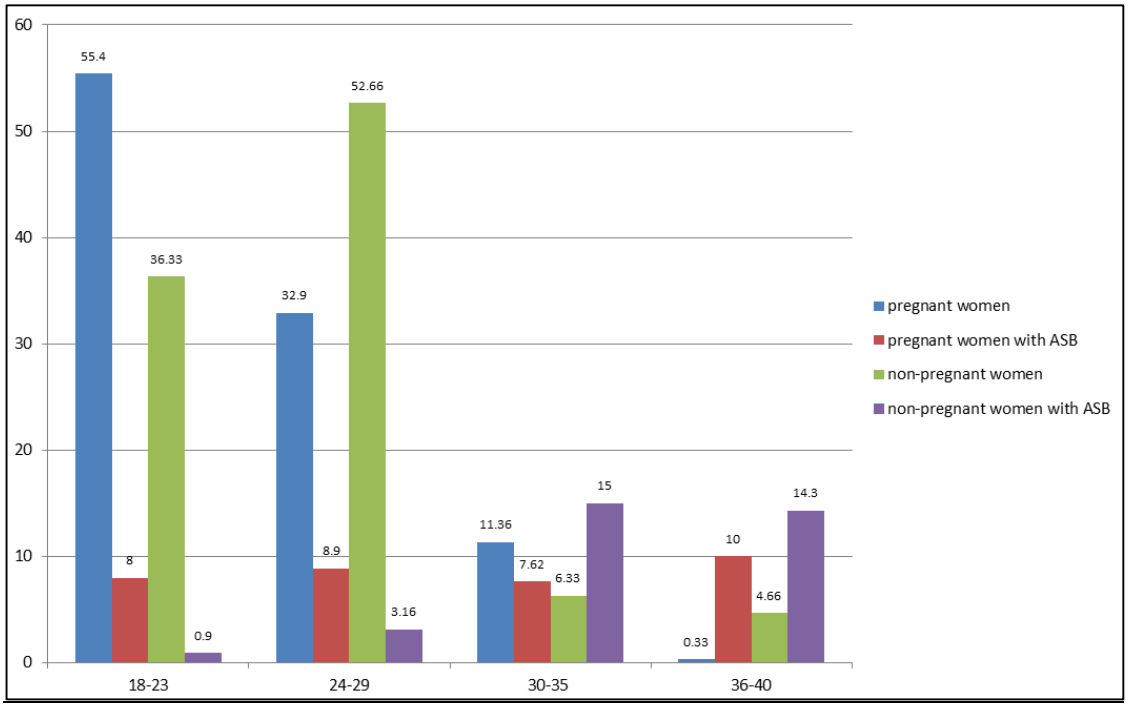
2.3 Collection and microbiological analysis

Collection and transportation of urine was carried out by standard procedures [7], microscopic examination by Wet film examination [8] and Gram staining using Hacker's modification [9], Culturing of uncentrifuged urine specimen was done by standard methods using growth promoting mediums for presence or absence of haemolysis and lactose fermentation, Culturing of centrifuged urine specimens was done on HiCrome UTI agar, Mac Coney's agar, Blood agar, Nutrient agar using standard loop technique 0.01ml of urine using surface streak calibrated loop technique [9], If growth was seen colonies were counted and these count was multiplied with 100 (since 0.01ml loop was used) to count colony forming unit (cfu) more than or equal to 10^5 bacteria/ml of one or two organisms on two clean catch cultures in absence of symptoms were considered for significant bacteriuria. Identification of the isolate was done on the basis of morphological, cultural characteristics and Rapid Biochemical Identification Kit. *E. coli strains* were identified by motility and KB003 Hi25^{TM} Enterobactriaceae Identification Kit [10].

2.4 Antibiotic sensitivity testing

AST of the *E. coli* isolates obtained from urine samples was carried out by Kirby - Bauer disk diffusion method [11], For total 111 *E. coli* isolates, antibiotics used were Ampicillin(10), Cephalexin(30), Nalidixic acid(30), Norfloxacin (10), Nitrofuratoin (300), Cotrimoxazole) (1.25 + 23.75), Augmentin(30), Ceftriaxone (30), Cefotaxime(30), Piperacillin +Tazobactum (100 + 10), Cefuroxime (30), Amikacin (30), Piperacillin (100). Value in bracket indicates antibiotic content in disc in mcg/ml. Resistance was ascertained by considering the break point MICs as per the norms.

3 Results

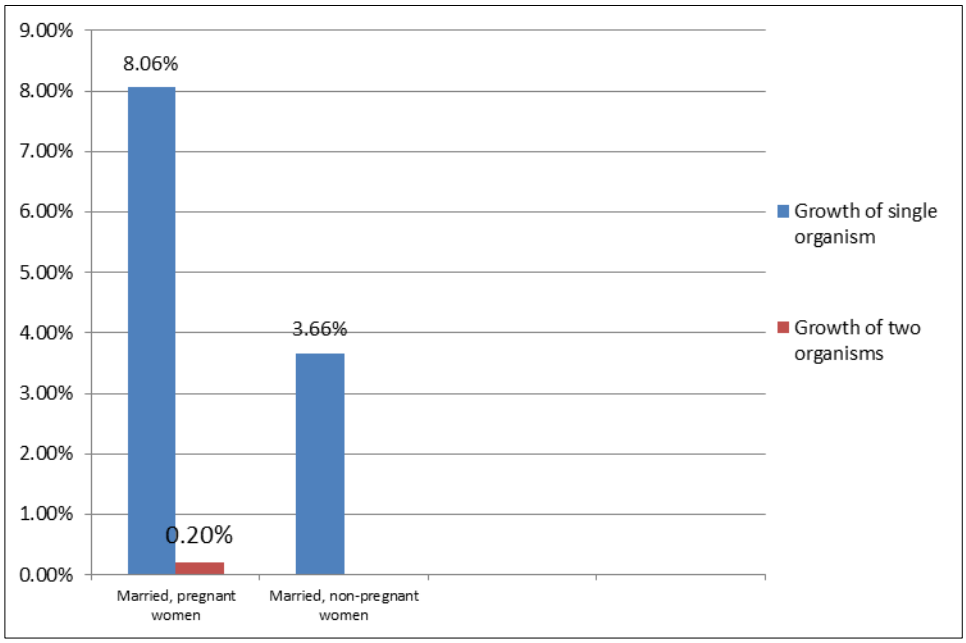


Married pregnant women =3000 Married non-pregnant women = 300

Graph 1: Age distribution of population studied for ASB (%)

In pregnant women, 55.4%, 32.9%, 11.36% and 0.33% of cases were in age group of 18- 23,24-29, 30-35 and 36-40 years respectively. While ASB was shown by 8%, 8.9%, 7.62% and 10% cases in age group of 18- 23,24-29, 30-35 and 36-40 years respectively.

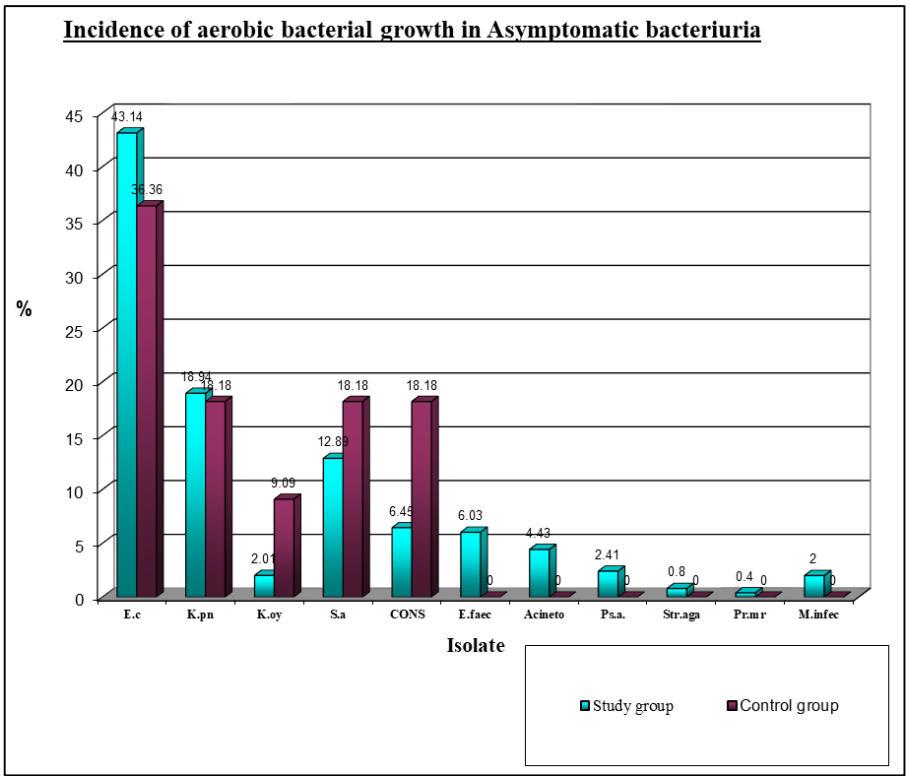
In the non-pregnant women group, 36.33%, 52.66%, 6.33% and 4.66% of cases were in age group of 18- 23,24-29, 30-35 and 36-40 years respectively. While ASB was shown by 0.9%, 3.16%, 15.00% and 14.3% cases in age group of 18-23, 24-29, 30-35 and 36-40 years respectively.(p value < 0.0001)



Married pregnant women =248 Married non-pregnant women = 11

Graph 2: Culture findings in study and control population with ASB

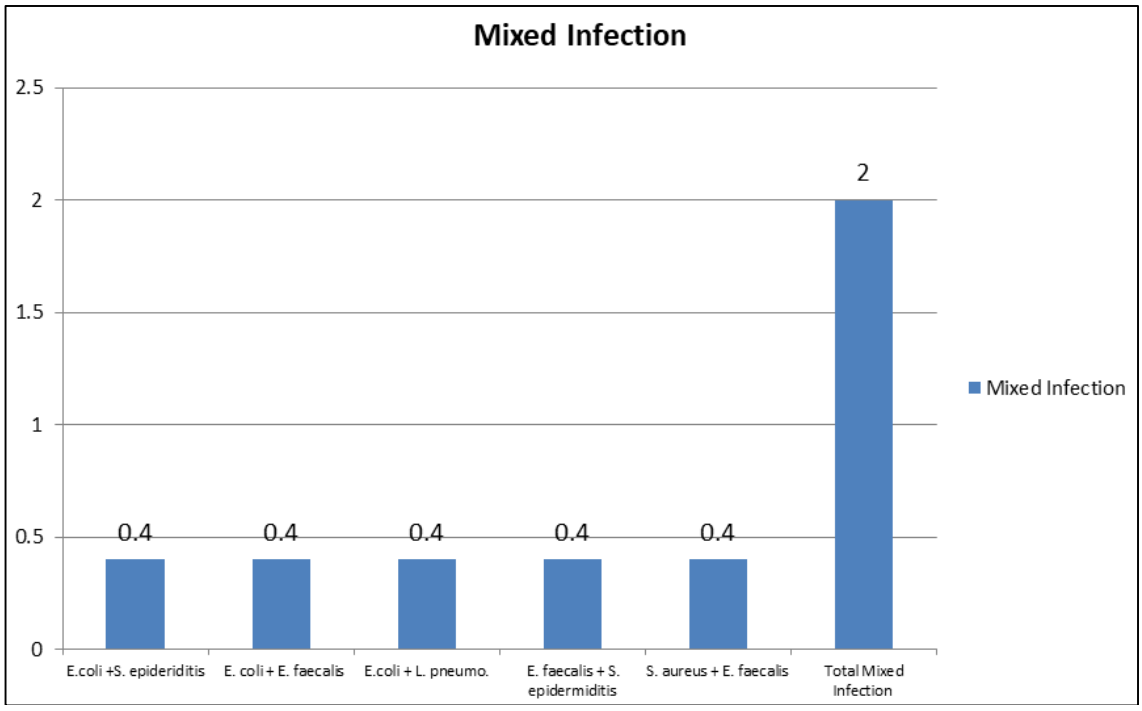
Prevalence of ASB was observed 8.26% and 3.66% in study & control group respectively. ASB is twice more common in pregnant than compared with non-pregnant women. p value < 0.0001. In pregnant women category, single pathogen was be detected in 8.06% cases, while 0.2% cases showed mixed infection of two pathogens. In non-pregnant women single pathogen was observed in 3.66% cases and no mixed infection is observed,



Married pregnant women =248 Married non-pregnant women = 11

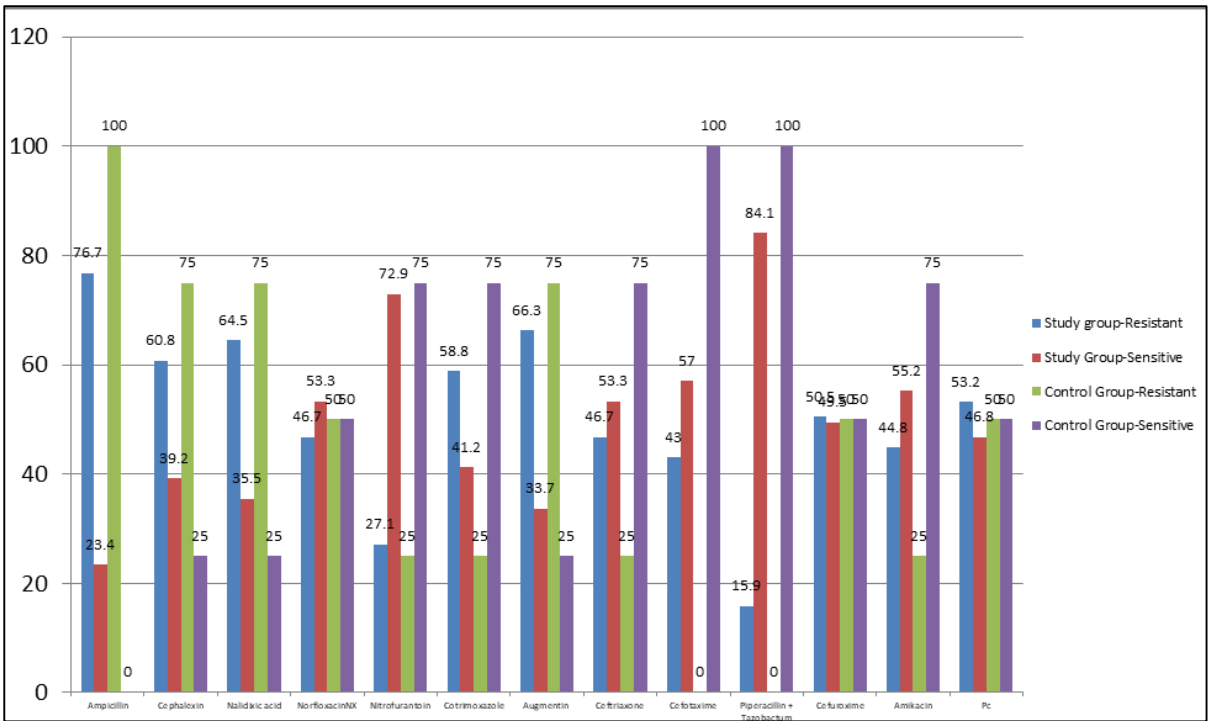
Graph 3: Isolates found in study and control group with ASB

In married pregnant women *E. coli* (E. c) was the predominant isolate (43.14%) followed by *Klebsiella pneumoniae (K.pn)* (18.94%), *Staphylococcus aureus* (S. a) (12.89%) and least was seen *Proteus mirabilis (Pr. mr)* (.4%). In this group mixed infection of two pathogens was detected. While in married non- pregnant women, *E. coli* was the predominant isolate (36.36%) followed by *Klebsiella pneumoniae* (18.18%) and CONS (18.18%) and least was seen *Klebsiella oxytoca (K.oy)* (0.4%). In this group *Enterococcus faecalis (E. faec), Acinetobacter baumanii (Acineto), Pseudomonas aeruginosa (Ps. a), Streptococcus agalactiae (Str. aga), Proteus mirabilis (Pr. mr)* and mixed infection was not detected. Still, etiology of bacterial agents is similar in both pregnant as well as non-pregnant women.



Graph 4: Microbiological findings of mixed culture in study group. (%) n=248

Mixed culture infection was found in study group (2%). In most the mixed infections *E. coli* was observed as one of the pathogen.



Graph 5: Antibiotic susceptibility testing of all *E. coli* isolates (n=111)

Among the *E. coli* isolates, 84% were sensitive Piperacillin + Tazobactum followed by 73% sensitivity to Nitrofurantoin. Around 50% of these strains were found to be sensitive to Norfloxacin, Ceftriaxone, Cefotaxime and Amikacin. Of these resistant was found amongst Ampicillin, Augmentin, Nalidixic acid, Cephalexin, and Cefuroxime. Almost picture was observed in *E. coli* isolates obtained from control group

4. Discussion

Humans normally produce 1 to 2 liters of urine per day, which must pass through bladder, corresponding to average flow rates of 40 to 80 ml per hour. An adult human bladder has a volume of 200 to 400 ml, and micturition causes release of roughly same volume of urine; the volume of urine remaining in bladder following micturition is ~1 ml [12]. The contribution of bladder hydrodynamics on elimination of bacteria has been recognized for several decades, and it has been suggested that without adhesin-assisted attachment to bladder surface, *E. coli* would not be able to overcome losses caused by micturition and, therefore, it would be unable to establish in UT. Implicit in this suggestion is the notion that the growth rate of *E. coli* in urine is too slow to cope with the losses incurred by micturition. Meanwhile, mathematical modeling suggests that if the growth rate of a strain is high enough it will be able to establish in the bladder in an adhesion-independent manner. Indeed, a theoretical analysis of bacterial growth in the bladder suggested that many urinary tract isolates of *E. coli* had growth rates that were fully compatible with no adhesive establishment in the bladder [12].

During pregnancy UTIs are high potential risk for mother and child, Majorities of UTIs are asymptomatic and places mother at risk for low birth weight and preterm birth. Main objective of study was to determine incidence and etiology in patients suffering with ASB with special reference to *E. coli* isolates infection.

Our study showed correlation of age and ASB cases in study and control group. Difference between them was statistically significant [5]. *Nath G et al, 1996* reported incidence among 24-29 years as 10.37% followed by 18-23years (5.26%) and >30years age group (12.43%). In our study, incidence of ASB in pregnancy was observed to be 8.26% which was well within range of earlier reports (5-10%) from India. While in non-pregnant women, ASB were 3.66%. These findings were similar to observations made by *Lavanya SV et al, 2002* [14], Nath G *et al*, 1996 [5] reported higher incidence of UTI in pregnancy (9.04%). *Bandyopadhyay S et al, 200* [15] reported incidence of ASB in pregnancy 4.34%. Prevalence of ASB in Libya, in all women was 8.3%, in pregnant women was 11.7%, and in non-pregnant women was 5%, This indicates, 16.7% of pregnant women were at risk of development of acute episode of UTI during pregnancy if they were not properly treated. Study in Libya (16) found significantly high relation between age & Bacteriuria and revealed, Bacteriuria in women was commonest in age group of 25-30 years (62.5%) and these result agreed with study by Buzayan MM in Libya [17], but contrast with study in Yemen, that observed Bacteriuria was more in age group of 15-24 years (53.7%) [18]. this difference may be due to social factors like early age of marriage and sexual activity.

In our study, anaerobic studies were avoided, as incidence of anaerobes is very low in UTI. Pattern of aerobic bacterial isolates obtained here was same in both groups and were identified in majority (8.26%) of pregnant women. [5] *Nath G et al, 1996* had reported 9.04% while *Lavanya SV et al, 2002* [14] reported 8.4% in pregnant women. Only 2.41% was more than two pathogens concomitant mixed infections. In our study group, *E. coli* was predominant organism accounting for 43.14%, followed by *K. pnumo.* (18.94%), *S.* aureus (12.89%), S. epidermidis (6.45%) and E. faecalis (6.03%). Acineto. & Ps. aero was found to be 4.43% and 2.41% respectively and <1% were Str. aga. And Pr. mir. According to Lavanya SV *et al* [14] 2002 *E. coli* was found to be in 83% cases, *E. coli* with Kleb. Species was in 4.7% cases, S. aureus with Kleb. Species was in 2.3% cases,. Nath G *et al*, 1996 [5] reported 30.6% of *E. coli* followed by 24.4% Coagulase negative Staphylococci (CONS), 20.4% of E. faecalis and 14.27% of S. aureus. It is quite evident that *E. coli* was most predominant causative agent of UTI followed by either Klebsiella or Pseudomonas species. *E. coli* and Klebsiella. Species being natural flora of intestine would cause UTI in individuals with poor hygienic conditions, particularly individuals who do not keep up the proper hygienic condition of anus and perianal region. A parallel study of control group, did not show any symptoms of UTI. Their urine samples were also cultured. Here, E. coli was predominant organisms (36.36%), followed by K. pneumo (18.18%), S. aureus and S. epidermidis (18.18%). E. faecalis, Acineto, Strep. agal. And Pr. mira and Ps. aero were not isolated here. In Libyan study [16] most frequent isolates were S. aureus (31.2%), *E.coli* (25%), S. saprophyticus(18.9%) etc, whereas another Libyan study found, Bacteriuria in pregnant women caused by *E.coli* was 65.5% and K. pneumo. 20.7% [17] and Al Haddad AM *et al* [18] found *E. coli* was most frequently isolated 41.5%, followed by S. aureus 19.5%, Study by V.P. Sarasu VP and Ramalatha SR (2017) [19] observed in Thanjavur, among the 150 pathogens isolated from the samples, the Gram Negative Bacilli (GNB) with 129 isolates (86.0%) was the major cause for UTI while only 21 isolates were Gram positive cocci (GPC). Among the 129 GNB, the *Escherichia coli* and Klebsiella spp alone constituted 77% of total isolates with 85 isolates of *E. coli* and 30 isolates of Klebsiella spp. Some of the international and national studies shows the % of *E. coli* they had observed. Sana *et al* in 2005 [20] found in Isreael 94%, in USA 90 %, Kuwait 90 %, England 75%, Sweden 74%, Italy 69%, Japan 65%, while Al-Haddad AM *et al*, 2005 [18] in Libya found 41.5%, Mouse *et al*. (2015) [21] found in bangala Desh 48.1%. Chatterjee *et al*. (2009) [22] found 90 %, 83.4% in Kashmir and Maharastra respectively, Baby Padmini and Appalaraju (2004) [23] in tamilnadu 49.3%, Mohanty *et al*. (2005) [24] in New Delhi 46%, Ghadage *et al*. (2016) [25]

In Pune 41.3%, V.P. Sarasu VP and Ramalatha SR (2017) [19] in Tanjavur 57% and our study observed 41.3% in pregnant women, Our study observed, among *the E. coli* isolates, 84% were sensitive Piperacillin + Tazobactum followed by 73% sensitivity to Nitrofurantoin. Around 50% of these strains were found to be sensitive to Norfloxacin, Ceftriaxone, Cefotaxime and Amikacin. Of these resistant was found amongst Ampicillin, Augmentin, Nalidixic acid, Cephalexin, and Cefuroxime. Almost similar picture was observed in *E. coli* isolates obtained from control group. While study by V.P. Sarasu VP and Ramalatha SR, (2017) [19] observed, the most effective antibiotics against all isolates were imipenem (100%) followed by amikacin

(84%), levofloxacin (83%), cefepime (81%), cefoxitin (76%), nitrofurantoin (61%) and ciprofloxacin (48%). Both ampicillin and co-trimoxazole were highly resistant shows only 11% and 13% sensitivity. Studies from USA, Europe and most other countries have shown better susceptibility pattern for pathogens isolated from UTI against co-trimoxazole [26].

But, in this region of the world co-trimoxazole has shown poor activity. A reason for this lack of sensitivity may be that in the past, co-trimoxazole has been extensively used in this region. Among the 85 *E. coli*, 70 (82.4%) strains were resistant to co-trimoxazole. Hence, co-trimoxazole cannot be recommended as an empiric therapy for the treatment of UTI in India. First, second and third generation cephlosporins, nalidixic acid and norfloxacin were resistant to all the isolated pathogen by 50% and more [19].

However, Akram *et. al*. (2007) [27] from India have reported 100% activity of imipenem against *E. Coli* and similar findings were also reported by Ullal *et. al*. (2009) from Pakistan [28].

Importance of Diagnosis of ASB has to be considered. Bacteria originate from the large bowel and colonize in the urinary tract transperineally. The most common infecting organism is *E coli*, which is responsible for 75-90% of Bacteriuria during pregnancy. Other organisms that have been isolated are Klebsiella, Proteus, Coagulase Negative Staphylococcus and Pseudomonas, It is important to identify and treat the infected group, as 40% of the ASBs develop acute symptomatic UTI, A positive history of previous UTI may be almost as effective as screening, in predicting UTI in pregnancy, Also, there is a good evidence of an association between any type of UTI in pregnancy and sudden unexpected infant death [29]. Relapse of UTI is the recurrence of Bacteriuria caused by the same organism, usually within 6 weeks of the initial infection. Reinfection is the recurrence of Bacteriuria with a different strain of bacteria, after successful eradication of the initial infection [31]. Approximately 15% of the patients will have a recurrence during pregnancy and a second course of treatment should be given, based on repeat culture with sensitivity testing.

E. coli was the most predominant organism in [31] one study 32 (51.61%), as reported in various other studies [32]. Numerous previous studies have established that the gold standard method for the diagnosis of UTI, as well as ASB, is the urine culture of midstream catch urine, It is well known that various other routine screening tests can only poorly detect all culture positive Bacteriuria cases in pregnant women [32]

It is well known that ASB indicates the active multiplication of bacteria in the urinary tract and 25% of the affected women are likely to develop acute pyelonephritis in the third trimester, if left untreated. Postpartum investigation is indicated when the UTI is recurrent, the incidence of ASB varies from 2-10%, depending on the socioeconomic status of the patients [33]. In one antenatal study, in which 9.9% of women took part in at least one screening, the risk of onset of Bacteriuria was highest between the 9th and 17th weeks of gestation. The 16th week is the optimal time for a single screen for bacteriuria, which has been calculated, based on the numbers of Bacteriuria free gestational weeks gained by the treatment [34],

5. Conclusion

E. coli (41.3 %) was the principal pathogen in pregnant and non-pregnant women. Study indicates a high resistance to most commonly used antibiotics due to indiscriminate use of antibiotics. Thus in order to prevent development of resistance, antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications. Institutional Antibiotic policy can be tailored to achieve superior therapeutic outcome. [19] Screening for ASB in all three trimesters is necessary to prevent the dangerous complications which are associated with ASB.

6. Acknowledgement

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