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Original Article

Phylogenetic background of *E. coli* isolated from asymptomatic pregnant women from Kolkata, India

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Abstract

Introduction: Asymptomatic bacteriuria (ABU) in pregnancy generates medical complications. *E. coli* is the common etiologic agent responsible for ABU-associated infections. This study aimed to identify the phylogenetic background and drug resistance in asymptomatic *E. coli* from a pregnant population.

Methodology: *E. coli* was confirmed biochemically from culture-positive urine samples collected from asymptomatic pregnant women. Phylogenetic typing was done by polymerase chain reaction (PCR). The isolates were subjected to antibiotic susceptibility testing and extended-spectrum beta-lactamase (ESBL) production. Statistical significance was determined using SPSS 17.0 software.

Results: Bacteriuria was observed in 113 (22.6%) of 500 asymptomatic pregnant females. *E. coli* was reported in 44 (38.9%) of 113 isolates. The mean age-wise distribution was 25.14 ± 4.63. Although pathogenic phylogroup B2 was predominant (54.5%), incidence of non-pathogenic phylogroup B1 (27.3%) was found to be statistically significant ($p \leq 0.001$), and B1 and B2 were correlated with respect to total ABU population. Antibiotic sensitivity against ampicillin (34.1%), ceftazidime (50%), cefotaxime (47.7%), ciprofloxacin (47.7%), amikacin (86.4%), nitrofurantion (79.5%), and co-trimoxazole (36.4%) was observed. Multidrug resistance (MDR) and ESBL production was reported in 26 (59.1%) of 44 and 18 (69.2%) of the 26 MDR isolates, respectively. A significant distribution of phylogroup B1 ($p = 0.03$) with drug resistance was also observed.

Conclusions: This is the first study that reported significant incidence of non-pathogenic phylogroup B1 in asymptomatic *E. coli* with high incidence of MDR isolated from pregnant women in Kolkata, India. These varied resistance patterns present major therapeutic and infection control challenges during pregnancy.

Key words: asymptomatic bacteriuria; urinary tract infections; uropathogenic *E. coli*, phylogenetic background.

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Introduction

Urinary tract infections (UTIs) represent the most common bacterial infection in pregnancy and are classified as either asymptomatic or symptomatic. Asymptomatic bacteriuria (ABU) is defined as the presence of significant bacteriuria without the symptoms of an acute urinary tract infection, and it can lead to symptomatic infection and subsequent adverse effects on pregnancy continuation [1]. Recently, it has been reported that UTIs usually develop in the third trimester [2]. The susceptibility to UTIs during this period may be due to urethral dilatation, which is maximal in the 22nd–24th weeks of gestation [3]. Untreated bacteriuria in pregnancy can lead to serious obstetric complications and poor maternal and perinatal outcomes, such as intrauterine growth restriction, preeclampsia, caesarean delivery, and preterm delivery [1,4]. Women with asymptomatic bacteriuria during pregnancy – which can lead to acute respiratory distress, transient renal failure, sepsis, and shock during pregnancy – show a 20- to 30-fold increased risk of developing pyelonephritis compared with women without bacteriuria [4]. Urine culture is recognized as the golden standard for detecting ABU showing bacterial growth > 10^5 colony-forming units (CFU)/mL. Therefore, all pregnant females should be screened for asymptomatic bacteriuria during their pregnancy, especially in their third trimester, as most cases of ABU (40%) are reported during this period of pregnancy; screening must be considered as a standard of obstetrical care that will help to minimize UTI-associated complications [2,5].

Escherichia coli have been shown in previous studies to be the dominant bacterial agents of ABU isolated from the urine of pregnant women [6,7]. ABU is commonly caused by strains of lower virulence than

the symptomatic uropathogenic *E. coli* strains, with a reduced frequency of virulence factor expression [8]. Only 22% of strains of *E. coli* isolated from women with asymptomatic bacteriuria had the capacity to adhere to uroepithelial cells, compared with 75% in the group of women who developed acute urinary tract infections [9]. *E. coli* isolates belong to four main phylogenetic groups: A, B1, B2, and D. Pathogenic isolates mainly belong to group B2 and, to a lesser extent, to group D, and most of the commensal or non-pathogenic strains belong to group A or group B1 [9,10]. ABU strains were phylogenetically related to strains that cause symptomatic UTIs, as previously reported in other ABU situations [1].

Emergence of MDR has also been reported [11], and poses risk in prescribing specific drugs to treat the infection. Moreover, there is no clear consensus in the literature on antibiotic choice or duration of therapy with bacteriuria in pregnant women, as drug resistance varies according to geographic location and may be related to misuse of antibiotics. Therefore, with increasing antibiotic resistance, considering local resistance rates must be considered when choosing therapy. This study was designed with the aim to determine the prevalence of *E. coli* in asymptomatic bacteriuria among pregnant woman from Kolkata, in eastern India, and to determine their phylogenetic background, antimicrobial resistance, and extended-spectrum beta-lactamase (ESBL) production.

Methodology

Isolation and identification of E. coli

A total of 500 urine samples collected from asymptomatic pregnant women were screened for significant bacteriuria. Of these, 113 urine samples yielded significant growth. Significant monomicrobic bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of > 10^5 CFU/mL. Only a single positive culture per patient was included in the analysis. *E. coli*were further confirmed by standard biochemical tests [12]. The media used during the study included MacConkey agar, Mueller-Hinton broth, and Luria Bertaini Broth (HiMedia, Mumbai, India). The cultures were incubated at 37°C for 24 hours. The *E. coli* isolates were sub-cultured in Luria Bertaini broth and maintained on MacConkey agar plates at 4°C for further study. The study protocol was approved by the institutional ethical committee.

Isolation of bacterial DNA

DNA for amplification was released from whole cells by boiling. Single colonies were harvested from the Luria Bertaini broth agar plates, suspended in 100 µL of sterile water, incubated at 100°C for 10 minutes, and centrifuged [13]. The supernatant was used in subsequent polymerase chain reaction (PCR).

Phylogenetic analysis

The distribution of phylogenetic groups among the isolates was determined by individual PCR using gene-specific primers against the three DNA markers (*chuA*, *yjaA*,and the DNA fragment *TSPE4.C2*). The size of amplicons obtained as PCR products allowed the *E. coli* isolates to be classified into one of the four major *E. coli* phylogenetic lineages: A, B1, B2, or D [14].

Antibiotic sensitivity

Susceptibility of isolates to different antibiotics were tested using the Kirby-Bauer disk diffusion method using Muller Hinton agar against selected antibiotics, namely ampicillin (AMP; 10 µg), ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), ciprofloxacin (CIP; 5 µg), amikacin (AK; 30 µg), nitroflurantoin (NIF; 300 µg), and co-trimoxazole (COT; 30 µg) (HiMedia). The sensitivity test was standardized using *E. coli* ATCC 25922 strain. Inhibition zone size was interpreted using standard recommendation of the Clinical Laboratory Standards Institute [15].

ESBL confirmatory test

All the *E. coli* isolates collected from the urine of hospitalized patients resistant to at least CAZ and/or CTX were subjected to ESBL confirmatory test using CAZ and ceftazidime-clavulanic acid (CAC), and CTX and cefotaxime-clavulanic acid (CEC) combination disks. A difference of 5 mm between the zone of inhibition of a single disk and in combination with clavulanic acid (inhibitor) was confirmed as an ESBL-positive isolate [15].

Statistical analysis

The data were analyzed using SPSS version 17.0. The Chi-square test was used to compare categorical variables. A p value of < 0.05 was considered to be statistically significant after Yates correction. Correlation was ascertained by calculating Pearson's correlation coefficient.

Results

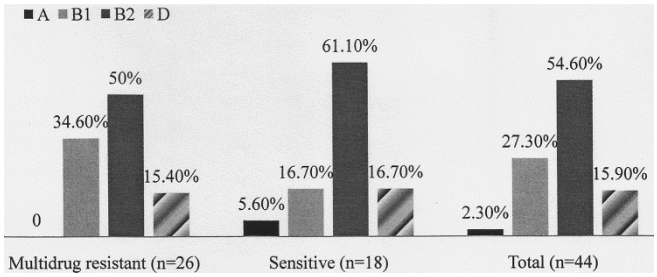
Occurrence of E. coli in asymptomatic bacteriuria

Bacteriuria was observed in 113 (22.6%) of 500 asymptomatic pregnant females. Biochemical analysis revealed that 44 (38.9%) of 113 asymptomatic isolates were *E. coli*. The mean age-wise distribution in women with *E. coli* associated ABU was 25.14 ± 4.63, the highest belonging to the 22–29 year age group (68.2%). This was closely followed by the 18–21 (15.9%) and 30–38 (15.9%) age groups.

Phylogenetic background and drug resistance

Overall predominance of the B2 (54.5%) followed by the B1 (27.3%) phylogroup was observed among the 44 asymptomatic *E. coli* isolates (Figure 1). Moreover, incidence of phylogroup B1 among the isolates was found to be statistically significant ($p \leq 0.001$), and B1 and B2 were correlated with respect to total ABU population. Table 1 summarizes the antibiotic sensitivity of the *E. coli* isolates against a spectrum of eight selected antibiotics of different classes: ampicillin (34.1%), ceftazidime (50%), cefotaxime (47.7%), ciprofloxacin (47.7%), amikacin (86.4%), nitrofurantion (79.5%), and co-trimoxazole (36.4%). Resistance against three or more than three groups of drug was designated as MDR [5]. Multidrug resistance and ESBL production was observed in 26

Figure 1. Phylogenetic background of the *E. coli* isolates associated with ABU. Prevalence of phylogroup A, B1 (non-pathogenic) and B2, D (pathogenic) among three subsets represented as multidrug resistant (n = 26), sensitive (n = 18) and total (n = 44) in percentage values.



(59.1%) of 44 and 18 (69.2%) of the 26 MDR isolates, respectively. Ten different resistant patterns were observed in the MDR isolates, with resistance against ampicillin, ceftazidime, cefotaxime, ciprofloxacin, and co-trimoxazole being predominant (Table 2). The ESBL producers were mostly sensitive to amikacin (15 of 18, 83.3%) and nitrofurantoin (12 of 18, 66.7%). Investigations on the prevalence of different phylogenetic groups among the multidrug-resistant and multidrug-sensitive isolates were A (0%, 5.6%), B1 (34.6%, 16.7%), B2 (50%, 61.1%), and D (15.0%, 16.7%), respectively (Figure 1). Furthermore, the statistical distribution of phylogroup B1 in drug-

Table 1. Antibiotic sensitivity among the asymptomatic *E. coli* isolates (n = 44)

Antibiotics	No. of asymptomatic *E. coli*, n (%)	
	Sensitive	Resistant
Ampicilin	15 (34.1)	29 (65.9)
Ceftazidime	22 (50)	22 (50)
Cefotaxime	21 (47.7)	23 (52.3)
Ciprofloxacin	21 (47.7)	23 (52.3)
Amikacin	38 (86.4)	6 (13.6)
Nitrofurantoin	35 (79.5)	9 (20.5)
Co-trimoxazole	16 (36.4)	28 (63.6)

Table 2. Drug resistance among the multidrug-resistant asymptomatic *E. coli* isolates (n = 26)

Resistance pattern	No. of isolates
AMP, CAZ, CTX, CIP, AK, NIT, COT	2
AMP, CAZ, CTX, CIP, AK, COT	4
AMP, CAZ, CTX, CIP, NIT, COT	4
AMP, CAZ, CTX, CIP, COT	6
AMP, CAZ, CTX, CIP	4
AMP, CIP, COT	1
AMP, CAZ, CTX, COT	2
AMP, CIP, COT	1
AMP, CAZ, CTX, NIT	1
AMP, NIT, COT	1

AMP: ampicillin (10 µg/disk); CAZ: ceftazidime (30 µg); CTX: cefotaxime (30 µg); CIP: ciprofloxacin (5 µg); COT: cotrimoxazole (25 µg); AK: amikacin (30 µg); NIT: nitrofurantoin (300 µg)

resistant isolates was significant ($p = 0.03$), and their distribution with respect to isolates belonging to phylogroup B2 was also significantly correlated.

Discussion

In this study, bacterial growth was observed in 22.6% of the asymptomatic pregnant women. The prevalence of asymptomatic UTIs has been previously reported to be 2% to 13% in pregnant women [5]. This variation may be explained by the fact that there were differences in the environments, social habits of the community, socioeconomic status, and standard of personal hygiene and education of the patients who were studied. Furthermore, *E. coli* was the predominant pathogen associated with asymptomatic UTIs in pregnant women globally [4,5, 10,13,16] as well as in India [11,17,18] and its sub-continent [6,19], as also observed in our study (38.9%). Earlier reports by Turpin *et al*. [20] and Akinloye*et al*. [21] revealed that advanced maternal age (≥ 35 years) was a risk factor for asymptomatic bacteriuria. However, other reports worldwide [4,16,19,22,23] and nationwide suggest a high incidence of ABU in pregnant women in the 20–30 year age group [7,11,17,18], consistent with our results, which also revealed 68.2% incidence of ABU in pregnant women in the 22–29 year age group, indicating that ABU is a risk factor for asymptomatic UTIs in pregnant women in this region of the country.

A better understanding of what differentiates ABU from symptomatic bacteriuria could be particularly helpful in monitoring patients who are unable to report symptoms, and it could help to avoid complications during pregnancy. This study indicated predominance of B2 phylotype in the *E. coli* isolates associated with ABU (Figure 1), as shown in a previous study [1]. However, significant incidence of non-pathogenic phylogroup B1 in the isolates investigated ($p < 0.001$) indicated association of isolates belonging to B1 phylogenetic origin in asymptomatic infection from this region in India.

In the present study, varied antibiotic resistance patterns were observed in the drug-resistant isolates, with resistance against ampicillin, ceftazidime, cefotaxime, ciprofloxacin, and co-trimoxazole being predominant. The isolates were highly sensitive to amikacin (86.4%) and nitrofurantoin (79.5%) (Table 1). These results were mostly in concurrence with other studies [11,17]. Emergence of resistant ABU strains has also been reported [16]; these observations may be due to indiscriminate use of the antibiotics. Incidence of ESBL production among the MDR asymptomatic *E. coli* isolates was highly alarming and justifies the importance of routine screening for significant bacteriuria in pregnant women. Therefore, urine culture followed by an antimicrobial sensitivity test should be the method of choice for screening for ABU to prevent maternal and perinatal morbidity. There is no clear consensus in the literature on either the duration of therapy or the choice of antibiotic for bacteriuria in pregnancy. Increasing antibiotic resistance complicates empirical regimens, and local resistance rates need to be taken into consideration when deciding on therapy.

While it is generally assumed that virulence gene decay in ABU and bacterial adaptation to different infected hosts reflect the host response to infection, reduction in virulence potential may be related to the phylogenetic origin of the microbes. Our results reveal significant prevalence of phylogroup B1 ($p \leq 0.001$)) in the asymptomatic *E. coli* isolates. Moreover, distribution of phylogroup B1 and B2 in the MDR isolates was significant ($p = 0.03$ and $p \leq 0.001$, respectively) but in the drug-sensitive isolates, only B2 was significant ($p \leq 0.001$). Therefore, these results indicate an association of the non-pathogenic phylogroup and drug resistance in the asymptomatic *E. coli* isolated from pregnant women in Kolkata.

Conclusions

The prevalence of MDR and ESBL-producing *E. coli* isolates associated with ABU in pregnant women from Kolkata, India, indicates that drug resistance among ABU is on the rise, and selection of appropriate antibiotics (after antibiotic susceptibility testing) is important and has to be addressed by policymakers in order to formulate a strict and safe antibiotic prescription policy for pregnant women to treat ABU-associated infection of the urinary tract. This report also, for the first time, shows a significant distribution of *E. coli* belonging to the non-pathogenic phylogenetic group B1 associated with ABU in pregnant women in Kolkata, India.

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References

1. Schnarr J, SmaillF (2008) Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy.Eur J Clin Invest 38: 50-57.
2. Gayathree L, Shetty S, Deshpande SR, Venkatesha DT (2010) Screening for asymptomatic bacteriuria in pregnancy: An evaluation of various screening tests at the Hassan District Hospital, India. J ClinDiagn Res 4: 2702-2706.
3. Hamdan HZ, Ziad AH, Ali SK, Adam I (2011) Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. Ann ClinMicrobiolAntimicrob 10: 2-12.
4. Moghadas AJ, Irajian G (2009) Asymptomatic Urinary Tract Infection in Pregnant Women. Iranian J Pathol 4: 105-108.
5. Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, Agegn A (2012) Bacterial profile and drug susceptibility Northwest Ethiopia pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital. BMC Res Notes 5: 197-203.
6. Marahatta R, Dhungel AB, Pradhan P, Rai SK, Choudhuri DR (2011) Asymptomatic bacteriuria among pregnant women visiting Nepal Medical College Teaching Hospital, Kathmandu, Nepal. Nepal Med Coll J 13: 107-110.
7. Kerure SB, Surpur R, Sagarad SS, Hegadi S (2013) Asymptomatic bacteriuria among pregnant women Int J Reprod Contracept Obstet Gynecol 2: 213-216.
8. Salvador E, Wagenlehner F, Köhler CD, Mellmann A, Hacker J, Svanborg C, Dobrindt U (2011) Comparison of asymptomatic bacteriuria *Escherichia coli* isolates from healthy individuals versus those from hospital patients shows that long-term bladder colonization selects for attenuated virulence phenotypes. Infect Imm 80: 668-678.
9. Mabbett AN, Ulett GC, Watts RE, Tree JJ, Totsika M, Ong CL. Wood JM, Monaghan W, Looke DF, Nimmo GR, Svanborg C, Schembri MA (2009) Virulence properties of asymptomatic bacteriuria *Escherichia coli*. Int J Med Microbiol 299: 53-63.
10. Lavigne JP, Boutet-Dubois A, Laouini D, Combescure C, Bouziges N, Mares P, Sotto A (2011) Virulence Potential of *Escherichia coli* Strains Causing Asymptomatic Bacteriuria during Pregnancy, J Clin Microbiol 49: 3950-3953.
11. Sujatha R, Nawani M J (2014) Prevalence of Asymptomatic Bacteriuria and its Antibacterial Susceptibility Pattern Among Pregnant Women Attending the Antenatal Clinic at Kanpur, India. J ClinDiagn Res 8: 1-3.
12. Myer, Koshi (2001) Methods in biochemical identification of bacteria. Myer's and Koshi's manual of diagnostic procedures in medical microbiology and immunology/ serology, 2nd ed. Department of Clinical Microbiology, Christian Medical College, Vellore, All India Press 195-202.
13. Farshad S, Emamghorashi F (2009) The prevalence of virulence genes of E. coli strains isolated from children with urinary tract infection. Saudi J Kidney Dis Transpl 20: 613-617.
14. Clermont O, Bonacorsi S, Bingen E (2000) Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol 66: 4555-4558.
15. Clinical and Laboratory Standards Institute (2010) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21. Wayne, PA: CLSI.
16. Tadesse E, Teshome M, Merid Y, Kibret B, Shimelis T (2014) Asymptomatic urinary tract infection among pregnant women attending the antenatal clinic of Hawassa Referral Hospital, Southern Ethiopia. BMC Res Notes 7: 155-159.
17. Girishbabu RJ, Srikrishna R, Ramesh ST (2011) Asymptomatic bacteriuria in pregnancy. Int J Biol Med Res 2: 740-742.
18. Lata RC, Kanga A, Thakur K, Kiran KM, Sood A, Chauhan S (2012) Prevalence of Pregnancy Associated Asymptomatic Bacteriuria: A Study Done in a Tertiary Care Hospital. J ObstetGynecolInd 62: 511-514.
19. Parveen K, Momen A, Begum AA, Begum MJ (2011) Prevalence of urinary tract infection during pregnancy. Dhaka National Med CollHos 17: 8-12.
20. Turpin CA, Minkah B, Danso KA, Frimpong EH (2007) Asymptomatic bacteriuria in pregnant women at-tending antenatal clinic at Komfoanokye teaching hospital, Kumasi. Ghana Med J 41: 26-29.
21. Akinloye O, Ogbolu DO, Akinloye OM, Terry Alli OA (2006) Asymptomatic bacteriuria of pregnancy in Ibadan, Nigeria: a reassessment. Br J Biomed Sci 63: 109-112.
22. Imade PE, Izekor PE, Eghafona NO, Enabulele OI, Ophori E (2010) Asymptomatic bacteriuria among pregnant women. North Am J Med Sci2: 263-266.
23. Mokube MN, Atashili J, Halle-Ekane GE, Ikomey GM, Ndumbe PM (2013) Bacteriuria amongst Pregnant Women in the Buea Health District, Cameroon: Prevalence, Predictors, Antibiotic Susceptibility Patterns and Diagnosis. PLoSONE 8: e71086.

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