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Vaginal Carriage of Antibiotic Resistant *Escherichia coli* by Pregnant Women: A Concern for the Neonate

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Abstract

Background: We report the resistance pattern and plasmid profile of vaginal isolates of *Escherichia coli* isolated from asymptomatic pregnant women attending the outpatient department of a primary health care centre in a district of North east India.

Methodology: Antibiotic susceptibility was done by disc diffusion method and interpretation as sensitive, intermediate or resistant was done as per Clinical Laboratory Standards Institute's interpretive standards for *Enterobactereriaceae*. *E. coli* ATCC 25922 was used as the control strain. Phenotypic screening for extended-spectrum beta-lactamase was done using the phenotypic disc confirmatory test. Plasmid DNA was extracted as per manufacturer's instructions using commercially available kit. The plasmid band and size was estimated by comparison with a 1kb DNA marker.

Results: A total number of 40 *E. coli* isolates were obtained after screening 246 pregnant women. Reduced susceptibility to at least one antimicrobial was seen in 34 isolates (85%) of *E. coli*. Highest resistance was to cefotaxime (60%). Twelve isolates (30%) were found to be multidrug resistant (reduced susceptibility to antimicrobial drugs belonging to ≥3 classes). Seventeen (42.5%) isolates were ESBL producers of whom 9 were multidrug resistant (MDR). Plasmid DNA isolation was done for thirty seven of the isolates of whom 4 did not show any band. The number of plasmids varied from 1 to 5 per isolate. Plasmid size ranged from 1 kb to above 10 kb when compared to a 1 kb ladder.

Conclusion: This study demonstrates that drug resistant *E. coli* exists as colonizers in the genital tract of pregnant women.

Keywords: *Escherichia coli*; Vaginal isolates; Pregnant women; India; Drug resistance; Plasmid profiling

Introduction

Escherichia coli is a normal inhabitant of the vagina and is seen to colonize upto 20% of pregnant women [1-4]. These colonizing isolates can sometimes cause complications during pregnancy or can be transmitted to the neonate leading to neonatal infection [1,5-7]. Ability to colonize and cause infections has been attributed to the presence of several virulence genes in these isolates [3,7].Vaginal colonization with *E. coli* is reported as a risk for very low birth weight delivery and other perinatal complications [1]. Studies from worldwide have reported isolation of drug resistant *E. coli* among vaginal isolates of pregnant women [2,8,9]. Transmission of these resistant strains to the neonate can prove fatal in whom early detection is challenging and treatment options are limited. Outbreaks in neonatal wards and adverse outcome due to drug resistant *E. coli* infection have been reported [10,11]. Thus identification and elimination of these resistant strains at the maternal level can have an impact on the reduction of fatal outcome in neonates especially in developing countries where the neonatal mortality rate is high [12].

In this context a pilot study has been done to determine the resistance pattern and plasmid profile of *E. coli* colonizing the vagina of asymptomatic pregnant women attending the outpatient department of a primary health care centre in a district of North east India.

Methodology

Study setting

The study was carried out in the month of February 2014 and March 2014. Two hundred and forty six asymptomatic pregnant women attending an outpatient department of a primary health care centre in Dibrugarh for routine antenatal check up were included in the study. Dibrugarh is situated in the Northeastern region of India in the state of Assam. The State's total population is 31.17 million (2011 census), maternal mortality rate is 390 per 100000 live births as per SRS (sample registration system) bulletin (June, 2011) and infant

mortality rate is 58 per 1000 live births (SRS December, 2011) [13,14]. All included participants are at least 18 years of age and informed consent were obtained before collecting samples. Ethical clearance was obtained from Institutional Ethics Committee prior to initiation of the study (Table 1).

Antibiotic resistance pattern of the isolates (Intermediate/ Resistant)	No. of isolates with similar pattern	No. of isolates showing plasmids	No. of ESBL producers	MDR
TX	1	0	1	No
NAL	2	1*	None	No
AMP, NAL	2	2	None	No
AMP, CTX	3	2	1	No
CTX, NAL	2	2	None	No
GEN, AMK, NAL	1	1	None	No
AMP, CTX, DOX	2	2	None	No
AMP, CTX, NIT	4	4	2	No
AMP, CTX, GEN	2	2	1	No
MP, CTX, SXT	2	2	2	No
AMP, CTX, SXT,NIT	1	1	None	Yes
AMP, DOX, SXT, NIT	1	1	None	Yes
AMP, CTX, GEN,AMK	1	1	1	No
AMP, CTX, NAL, SXT	1	1	None	Yes
AMP, CTX, AMK, NAL	1	None	1	Yes
AMP, CTX,NIT, NAL	1	1	1	Yes
AMP, CTX, GEN,CIP, SXT	1	1	1	Yes
AMP, CTX, CIP, NAL, SXT	1	1	1	Yes
AMP, CTX,NIT, NAL, SXT	3	3	3	Yes
AMP, CTX, CIP, NAL, NIT, SXT	1	1	1	Yes
AMP, CTX, CIP,GAT, DOX, NAL,SXT	1	1	1	Yes

Table 1: Resistance pattern and plasmid profile of the vaginal E.coli isolates from pregnant women: *one isolates was not tested; AMP-Ampicillin; AMK-Amikacin; CTX-Cefotaxime; CIP-Ciprofloxacin; DOX-Doxycycline; GEN-Gentamicin; NAL-Nalidixic Acid; NIT-Nitrofurantoin; SXT-Sulphamethoxazole trimethoprim

Collection and transportation of sample

Vaginal swabs were collected using sterile cotton swab (Himedia, Mumbai) by a nurse under the supervision of the attending Gynaecologist without using a speculum from the lower vaginal wall [1]. The swabs were immediately placed into Stuart's transport media (Himedia, Mumbai) and transported to the laboratory at room temperature within 5-6 hours.

Identification of bacterial isolates

The transported samples were plated onto MacConkey agar (HiMedia, Mumbai) and incubated aerobically at 37˚C for 24 hours. The isolates were identified based on colony appearance, gram stain and standard biochemical tests [15]. All media for biochemical tests were obtained from HiMedia, Mumbai.

Antimicrobial susceptibility testing (AST)

AST was done by disc diffusion method on Mueller Hinton agar (MHA) (HiMedia, Mumbai) using following antimicrobial agents: ampicillin (10 µg), amikacin (10 µg), doxycycline (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), (HiMedia, Mumbai) cefotaxime (30 µg), gentamycin (10 µg), ciprofloxacin (5 µg), gatifloxacin (5 µg), trimethoprim-sulfamethoxazole (1.35/23.75 µg) (BD BBL Sensi-Disc, USA). Interpretation as sensitive, intermediate or resistant was done as per Clinical Laboratory Standards Institute (CLSI) interpretive standards for *Enterobactereriaceae* [16]. *E. coli* ATCC 25922 was used as the control strain.

Phenotypic screening for extended-spectrum beta-lactamase (ESBL): Isolates showing reduced susceptibility to third generation cephalosporins were phenotypically confirmed for ESBL using the phenotypic disc confirmatory test (PDCT). This test was performed as

a disc diffusion test, as recommended by the CLSI [16]. The test inoculum (0.5 McFarland's turbidity) was spread onto the MHA by using a sterile cotton swab, then a cefotaxime (CTX) disc containing 30 µg of the antibiotic and a cefotaxime/clavulanic acid (CEC) disc (HiMedia, Mumbai) containing 20+10 µg of the antibiotics were placed at a distance of 30 mm from each other. The plates were incubated overnight at 37ºC. A ≥5 mm increase in the zone diameter for CEC, versus its zone diameter when it was tested alone by CTX was confirmed an ESBL-producing *E. coli*.

Plasmid DNA extraction

E. coli was grown overnight in 10 mL of Luria Bertani broth (HiMedia, Mumbai) at 37˚C. Plasmid DNA was extracted using GeneJET Plasmid Miniprep kit (Thermo Scientific, Lithuania EU) as per manufacturer's instructions. The purified plasmid DNA was stored at -20˚C for further use.

Agarose gel electrophoresis of plasmid DNA

Electrophoresis was carried out in 0.6% agarose gel and stained with ethidium bromide. A 1 kb DNA ladder (Promega, USA) was used as a reference marker. The plasmid band and size was estimated by comparison with DNA marker.

Results

A total number of 40 *E. coli* isolates were obtained after screening 246 pregnant women. All the isolates were screened for susceptibility to antimicrobial agents. The resistance pattern of the *E. coli* isolates is shown in Table 2.

Antibiotic (No of isolates tested)	Susceptibility pattern		
	Sensitive	Intermediate	Resistant
	No (%)	No (%)	No (%)
Ampicillin (40)	12 (30.0)	5 (12.5)	23 (57.5)
Cefotaxime (40)	12 (30.0)	4 (10.0)	24 (60.0)
Gentamycin (40)	35 (87.5)	3 (7.5)	2 (5.0)
Amikacin (36)	33 (91.7)	3 (8.3)	-
Ciprofloxacin (40)	36 (90.0)	2 (5.0)	2 (5.0)
Gatifloxacin (38)	37 (97.4)	-	1 (2.6)
Doxycycline (14)	10 (71.4)	2 (14.3)	2 (14.3)
Nalidixic acid (39)	23 (59.0)	8 (20.5)	8 (20.5)
Nitrofurantoin (38)	27 (71.05)	3 (7.9)	8 (21.05)
Trimethoprim-sulfamethoxazole (38)	26 (68.4)	2 (5.3)	10 (26.3)

Table 2: Susceptibility pattern of Vaginal E.coli isolates from pregnant women to different antimicrobial agents F

Reduced susceptibility to at least one antimicrobial was seen in 34 isolates (85%). Twelve isolates (30%) were found to be multidrug resistant (reduced susceptibility to antimicrobial drugs belonging to ≥3 classes). Seventeen (42.5%) isolates were ESBL producers of whom 9 were multidrug resistant (MDR). Plasmid DNA isolation was done for thirty seven of the isolates of whom 4 did not show any band. Among the isolates the number and size of the plasmids varied. The number of plasmids varied from 1 to 5. Plasmid size ranged from 1 kb to above 10 kb when compared to a 1 kb ladder.

Discussion

Maternal colonization with *E. coli* is a risk for neonate and it is suggested that the vagina in pregnant women and the amniotic fluid are two barriers that favour the selection of a population of *E. coli* strains that present a high risk of infection for neonates [7]. The emergence of resistance among these colonising isolates can act as a reservoir for infections in the mother as well as the neonate influencing treatment options in them [9].

Isolates in the present study had shown highest resistance to cefotaxime (60%). The cephalosporin resistance observed may be a result of extensive use of this drug as observed by others [9]. Total resistance to cefotaxime among vaginal *E. coli* isolates from pregnant women in Iraq was documented in a recent study [9].

In Argentina resistance rate to ampicillin, gentamicin, and cefotaxime was found to be 48.6, 10.8, and 0.8%, respectively. Enterobacteriaceae resistant to third generation cephalosporins were recovered in 7.3% of all perianal specimens. Among them, 5.4% of pregnant women were colonized with *E. coli* ESBL producer strains [2].

Similar resistance reports are available from India. In a study from Central India 109 *E. coli* isolates out of 710 screened were ESBL producers and 35 were multi drug resistant. They concluded that antibiotic usage and history of hospitalization in the last 4 weeks as a risk factor for MDR *E. coli* carriage [8]. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories according to the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) [17]. In another Indian study about 75% of *E. coli* isolated from neonatal septicaemia were ESBL producers. Fatal neonatal meningitis due to ESBL producing *E. coli* has also been reported and confirmation of mother to neonate transmission was confirmed [8].

ESBL's are plasmid mediated and organisms producing such enzymes are also co-resistant to many other classes of antibiotics thus limiting therapeutic options [18,19]. Studies available have shown relation of number and size of plasmids with the resistance characteristics of *E. coli* [18,20-22].

The recommended initial empirical therapy for a neonate with suspected bacterial sepsis and/or meningitis includes ampicillin and an aminoglycoside such as gentamicin. In many settings cefotaxime is used in addition to or instead of gentamicin, particularly when Gram-negative infections are suspected. Thus the transmission of these resistant vaginal isolates to the neonates is of major concern. Among our isolates resistance to these antibiotics were observed.

This study demonstrates that drug resistant *E. coli* exists as colonizers in the genital tract of pregnant women. Further studies to find out the risk factors associated with carriage of these resistant strains are required. Comparison of these maternal isolates with that of those causing neonatal infections is required for establishing transmission so that prophylaxis can be initiated at the maternal level. It is seen that often treatment in resource-poor settings has to be initiated on empiric basis due to unavailability of culture results. This

may result in emergence of resistant strains. Thus studies done to provide the clinicians with a data on the most common organism and their susceptibility pattern in their settings could help them in their practice for prescribing sensitivity specific treatment thus reducing emergence of resistance.

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