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A Study to Detect ESBL producing *Escherichia coli* Isolates From Women with Genital Tract Infection

KEYWORDS: Aerobic Vaginitis, ESBL,Vaginal E.Coli.

Divya .G
Department of Microbiology, Shri Sathya Sai Medical College & Research Institute, Thiruporur, Sri Balaji Vidyapeeth University,Tamil Nadu, India.

Karthika Jayakumar

ABSTRACT *Colonization of E.coli in vagina is recognized as an extra intestinal manifestation, associated with various infections.Occurrence of ESBL producing E.coli has become major concern due to its limited therapeutic option.Aim of the study is to detect, ESBL producingE. coli isolated from women with symptomatic genital tract infection.A total of 200 High Vaginal Swabs were collected, among which significant growth of pathogens was detected in 185 samples. 47 samples were positive for E.coli. 53% of E.coliwas found to be resistant to 3G cephalosporins and all the 53%of resistant isolateswere detected as ESBL producers phenotypically.As there are only limited reportsavailable regarding the presence of ESBL producing E.coli in vagina of pregnant and non pregnant women in India. This study may be helpful for clinicians to be aware of the presence of ESBL-producing organisms in vagina and initialization of surveillance studies to ascertain this.*

INTRODUCTION:

Female genital flora contains wide variety of 5 to 15 different bacterial species, including aerobes and anaerobes. Gram negative facultative anaerobic *Escherichia coli* are one of the normal inhabitants of the vagina and are seen to colonize up to 20% of pregnant women. [1, 2]

In vagina, *Escherichia coli* are considered as important flora next to Lactobacillus spp, due to its non-specific immunity. Vaginal colonization by *Escherichia coli* was recognized as a source of extra intestinal [3] *E. coli* associated with various symptomatic infections such as vaginitis or tubo-ovarian abscess, genitourinary and obstetric complications such as the severe form of pelvic inflammatory Disease [4], urinary tract infections [5], very-low-birth-weight Infants, early-onset neonatal septicemia and meningitis [6] and sexually transmissible disease to a male partner [7].

In recent years *E.coli*, reported as a predominant organism in causing aerobic vaginitis. Aerobic vaginitis (AV) is a state of abnormal vaginal flora that is distinct from the more common bacterial vaginosis. It is caused by a displacement of healthy vaginal species with aerobic pathogens such as *Escherichia coli, Staphylococcus aureus,* Group B *Streptococcus.* Clinical signs and symptoms includes vaginal inflammation, an itching or burning sensation, dyspareunia, yellowish discharge, an increased vaginal pH > 4.5 and inflammation with leukocyte infiltration. In pregnant women it causes pregnancy complications, such as ascending chorioamnionitis, preterm rupture of the membranes and preterm delivery [8, 9, 10].

Extended-spectrum β-lactamases (ESBLs) are capable of hydrolyzing broad spectrum cephalosporins and monobactams. In addition, these organisms also exhibit co-resistance to many other classes of antibiotics including aminoglycosides, quinolones, and cotrimoxazole [11]. The increased incidence of ESBL producing strains of *E. coli* over the past few years becomes a worldwide problem resulting in limited therapeutic options.

Vaginal carriage of *E. coli* causes aerobic vaginitis represents a real threat especially in causing various symptomatic genital as well as neonatal infections. These conditions do not respond to the antibacterial vaginosis medication. However, there are only very few reports available regarding their antibiotic resistance in India, therefore this study was carried out to detect , ESBL producing *E. coli* isolated from vagina of women with symptomatic genital tract infection.

OBJECTIVES:

Isolation of *E. coli* from high vaginal swab of symptomatic pregnant and non-pregnant women. To determine the antibiotic susceptibility and resistance pattern of *E. coli.*

To detect ESBL producing *E.coli* by phenotypic confirmatory disc diffusion method (PCDD).

MATERIALS AND METHODS:

A study group includes women of reproductive age group with complaints of serous vaginal discharge, lower abdominal waist pain and itching. A total of 200 high vaginal swabs (HVS) were collected from pregnant and non-pregnant women attending obstetrics and gynaecology department of Shri Sathya Sai Medical College & Research Institute over a period of 10 months from April 2014 to January 2015.

Collected samples were subjected for microscopical observation and cultured on Mac Conkey agar, blood agar and chocolate agar. After 24 hours of incubation at 37 ° C the pathogenic organisms were identified by standard protocol [12].

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

Antimicrobial susceptibility testing was done on Muller Hinton agar by disc diffusion method using CLSI guidelines [13]. The antibiotic tested were Ampicillin (10µg), Cefazoline (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefipime (30µg), Imipenem (10µg), Gentamicin (10 µg), Amoxycillin/clavulanic acid (20/10µg), Amikacin (30µg) and Ciprofloxacin (5µg). Interpretation of the diameter of zone of inhibition was performed using CLSI guidelines [13].

ESBL DETECTION:

Phenotypic confirmatory disk diffusion (PCDD) method was used to confirm ESBL production. Lawn culture of the test organism was made and 3rd generation Cephalosporin,

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Ceftazidime (30µg) disc was tested alone and along with their combination for 10µg of Clavulanic acid. Organisms with 5mm increase in zone of inhibition for ceftazidime / clavulanic acid (30µg/10µg) were confirmed as ESBLs. [13]

RESULTS:

A total of 200 HVS were collected from pregnant and non-pregnant women patients. Of which significant growth of pathogenic organism was obtained in 185 samples accounting for 92.5%. The remaining 15 (9.5%) samples were Normal vaginal flora.

Out of 185 isolates 94 isolates (51%) were obtained from pregnant women and 91isolates (49.1%) were from non-pregnant women. Of which 76.2% of isolates were of gram negative bacteria and the remaining 23.7% of isolates were gram positive organisms and Candida sps.

Escherichia coli was the most predominant isolate (25.4%), followed by *Klebsiella pneumonia and K. oxytoca* (23.7%), *Pseudomonas aueroginosa* (14%), *Staphylococcus aureus* (12.4%), *Acinetobacter baumannii* (8.1%), Group B *Streptococcus* (5.4%), *Candida albicans* (3%), *Proteus mirabilis* (2.7%), *Enterococci sps* (2.7%), *Citrobacter* koseri (2%), *Micrococci sps* (0.5%). **(TABLE: 1).**

From the total of 185 isolates, 47 isolates were *E.coli*, of which 25 isolates showed reduced susceptibility to the third generation cephalosporins (3GCs) e.g. ceftazidime (30 µg) and cefotaxime (30 µg) by Kirby-Bauer disc diffusion method. 53% of *E.coli* was found to be Multi Drug Resistant (MDR) strains. **(TABLE: 2)(FIGURE: 1).**

All the 47 *E.coli* isolates were tested for ESBL production by phenotypic confirmatory disk diffusion test. Among which a total of 25 isolates accounting for 53% (40% and 68% of pregnant and non-pregnant women's isolates, respectively) showed positive for ESBL production phenotypically. Remaining 22 (46.8%) were negative for ESBL production in in-vitro test **(TABLE: 3) (FIGURE: 2&3)**

TABLE: 1 Frequency of occurrence of bacterial isolates in HVS samples obtained from pregnant and non-pregnant women

S. No	Organisms	Number of isolates (n=185)	Pregnant Women (n=94)	Non-pregnant Women (n=91)
1	Escherichia coli	47	25	22
2	Klebsiella pneumonia	30	20	10
3	Klebsiella oxytoca	14	10	4
4	Pseudomonas aeuroginosa	26	13	13
5	Staphylococcus aureus	23	10	13
6	Acinetobacter baumannii	15	5	10
8	Group B Streptococcus	10	4	6
9	Candida albicans	5	1	4
10	Proteus mirabilis	5	1	4
11	Enterococci sps	5	1	4
12	Citrobacter sps	4	3	1
13	Micrococci sps	1	1	-
	Total	185	94	91

TABLE: 2 Antibiotic susceptibility pattern of *Escherichia coli.*

Serial no.	Antibiotics	Resistant (%)	Sensitive (%)
1	Amikacin	7(14.8%)	40(85.1%)
2	Gentamicin	10(21.2%)	37(78.7%)
3	Ciprofloxacin	4(8.5%)	43(91.4%)
4	Imipenem	0(0)	47(100%)
5	Cefazoline	25(53%)	22(46.8%)
6	Cefotaxime	25(53%)	22(46.8%)
7	Cefipime	25(53%)	22(46.8%)
8	Ceftazidime	25(53%)	22(46.8%)
9	Amoxycillin/ clavulanic acid	23(48.9%)	25(53%)

TABLE: 3 Result of ESBL detection in pregnant and non-pregnant women

ESBL	No of Isolates n= 47(%)	
	Pregnant women(n=25)	Non pregnant women(n=22)
Positive	10(40%)	15(68%)
Negative	15(60%)	7(31.8%)

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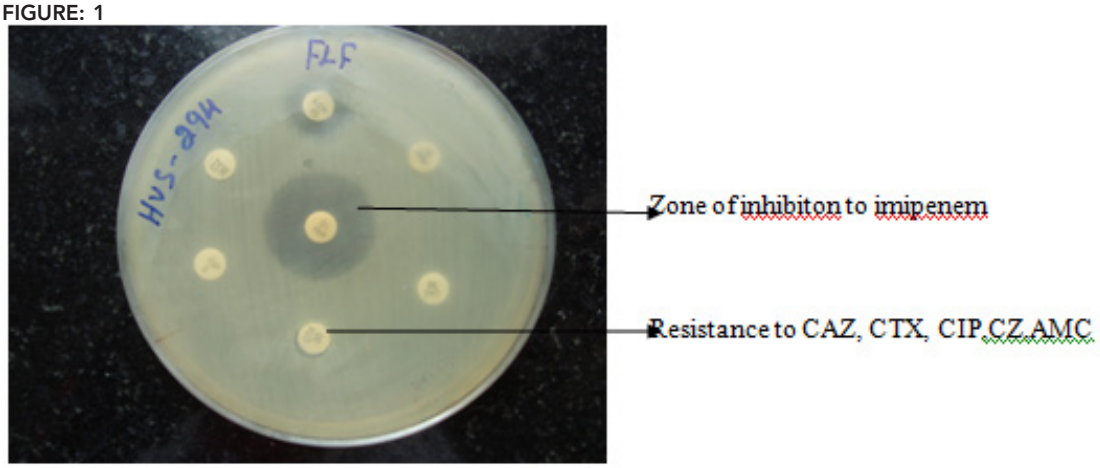
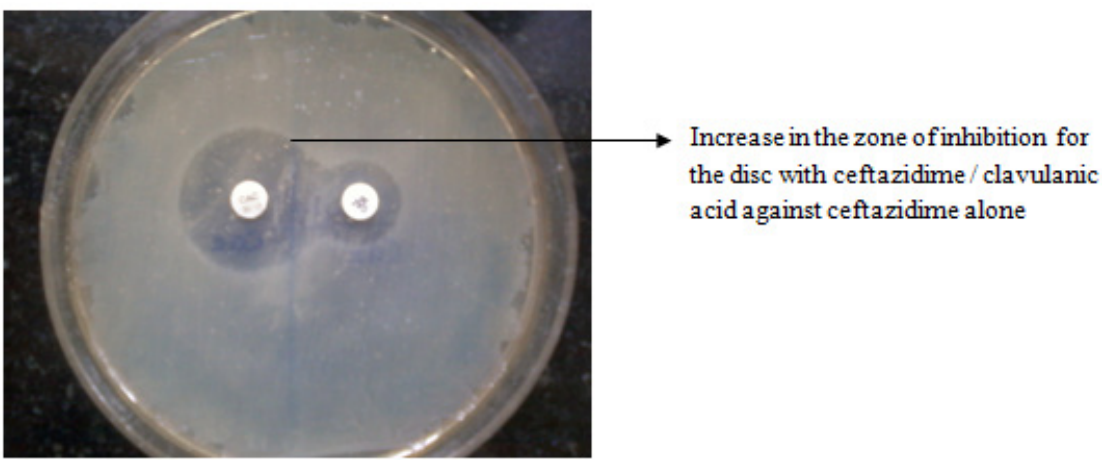
FIGURE: 1



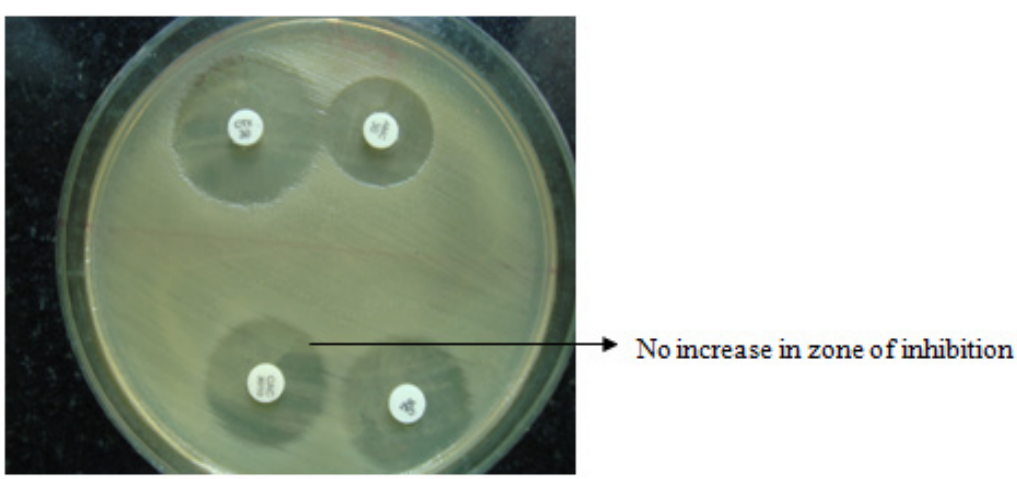
Kirby Bauer Disk Diffusion test Showing MDR pattern of *E.coli*

FIGURE: 2



Phenotypic confirmation disk diffusion (PCDD) showing ESBL producing *E.coli*

FIGURE: 3



Showing ESBL NEGATIVE *E.coli*

DISCUSSION:

Normal vaginal flora comprises of complex balance of organisms, the most predominant organism is *Lactobacillus sps*, which is mainly responsible for maintenance of the acidic vaginal pH, thereby gives protection from various pathogens which invades vaginal mucosa. But due to over-the counter usage of broad spectrum antibiotics like penicillin and tetracycline can suppress these protective bacteria resulting in colonization of drug resistant organisms in the genital tract causing bacterial vaginitis[14].

Our study includes female patients of reproductive age group with vaginal discharge diagnosed for bacterial vagi-

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nitis. In our study predominant organism isolated was gram negative aerobic and facultative anaerobic pathogens showed 76.2%. This was found to be high when compared to other study conducted in Iraq in which they have reported 38.2% of gram negative bacteria [15]. Similarly Saini [16] *et al.*, from India, also reported less 47.4% of Gram-negative bacteria in Pelvic Inflammatory Disease. But our study revealed higher percentage of isolation of gram negative bacteria from vagina which was considered as the predisposing factor for aerobic vaginitis.

In our paper the commonest gram negative bacterium isolated from the vagina of women with vaginitis was *E.coli* accounting for (25.4%). These result showed agreement with other report in India which also showed predominant isolate as *E.coli* [14].

Similarly Sareaa [17] *et al.*, from Iraq reported high frequency of *Escherichia coli* (57.5%). The occurrence of *E.coli* among pregnant and non-pregnant women in their study was 30 (56.6%) in non- pregnant women which was higher when compared to pregnant women 12 (60%). But in our study occurrence of *E.coli* was observed to be higher in pregnant women with 25(53%) than non-pregnant women 22 (46%).

The incidence of infection in pregnant women was found to be high in our study this could be attributed to hormonal changes, expanding uterus and constant stasis of urine during pregnancy [18] [19]. The occurrence of *E.coli* in vagina not always connected with UTI but the proximity of the short urethra allows ease of passage of pathogens from the vagina to the bladder [20]. In addition to this improper hygienic habits may also contributes to the colonization of *E.coli* which is a common inhabitant of large intestine [21].

In the present study 25 isolates of *E.coli* showed resistance to 3GC by Kirby Bauer disk diffusion method. The most effective antibiotics were Imipenem (0%), Ciprofloxacin (8.5%), Amikacin (14.8%) and Gentamicin (21.2%) which showed low percentage of resistance. Hence these antibiotics can be used as effective chemotherapeutic agents against ESBL producing isolates.

In our analysis 53% of *E.coli* was positive for ESBL production with 40% and 68% of pregnant and non-pregnant women. Similarly a report from Iraq [17] showed 56.2% of ESBL-producing isolates (50.0% and 61.1% of pregnant and non-pregnant women's isolates, respectively). However another report from Iraq [15] showed high prevalence of ESBL producing *E.coli* with 71.4%. But there are only limited data available on ESBL producing *E.coli* from vaginitis in India.

Vaginal colonization by ESBL-producing *E. coli* in women may provide future infections both in women and their contacts. The women themselves are vulnerable to acute and recurrent urinary tract infections with vaginal strains [22] as well as there is the possibility of transferring such strains to their sexual partners [7].

The nosocomial risk of vaginal colonization by such isolates may possibility create an outbreak in the hospital environment especially in the neonatal intensive care unit (NICU) as a result of the transfer of these isolates from the mother to the neonate during delivery[23]. Infection from such an extended spectrum β-lactamases (ESBLs) producers might be difficult to treat.

To our knowledge, there was no data on prevalence of ESBL producing *E.coli* in our setting. Hence this study would provide awareness for proper use of antibiotics and initiation of treatment for patients with drug resistant strains.

CONCLUSION:

Escherichia coli in vagina act as a reservoir for fecal-vaginal-urinary/neonatal route of transmission in extra intestinal *E.coli* infections. Our study reveals 53% of ESBL production among *E.coli* isolated from genital tract of pregnant and non-pregnant women. Existence of such MDR resistant strain in vagina may lead to various other complications like PID, Urinary tract infection and neonatal meningitis [20] [21][22]. As there are very limited data available in India regarding multi-drug resistance *E.coli* causing aerobic vaginitis. In conclusion, this study would be helpful for clinical microbiology labs and clinicians to be aware of the presence of ESBL-producing organisms in vagina and initialization of surveillance studies to determine this.

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