
ORIGINAL RESEARCH ARTICLE**Extended Spectrum Beta-Lactamase (ESBL) Producing
Uropathogens in Pregnancy**

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Abstract:**Aims & Objectives:**

1. To isolate the uropathogens from cases of asymptomatic bacteriuria in pregnancy.
2. Estimate rate of incidence of culture proven cases of asymptomatic urinary tract infection.
3. To carry out antimicrobial susceptibility testing of these isolates to commonly used antimicrobials & find out their ability to produce ESBL.

Methodology:

Urine sample was collected from antenatal women attending January 2018 to November 2018 pregnant women's attending antenatal clinic in BKL Walawalkar Rural Hospital at OBGY department were included in study. The recovered isolates which fit into inclusion criteria are first identified using standard bacteriological techniques and are subjected to antimicrobial sensitivity testing to commonly used urinary antimicrobials. The microorganisms isolated from these sample were also subjected to detection of ESBL production by standard disc diffusion testing method/ disc potentiation test.

Results:

A total of 180 pregnant ladies were enrolled during study period, urine sample was collected from them, out of this 180 samples, 33 (18.33%) were found to be culture positive cases of significant asymptomatic urinary tract infection. The different etiological agents were isolated during this study such as Escherichia coli (n=18), Klebsiella species (n=8), Pseudomonas aeruginosa, pseudomonas species, staphylococcus aureus and candida albicans. Antimicrobial susceptibility testing and

detection of ESBL production were carried out in these different isolates surprisingly we found 15 (54%) isolates were ESBL positive. It was observed in study that some antimicrobial agents like, Fosfomycin (92.85%), Amoxicillin clavulanic acid (89.28%) & Cefuroxime (60.71%), Ceftazidime (57.14%), Cefotaxime (53.71%) & Nitrofurantoin (50%) were showed high resistance pattern in vitro.

Conclusion:

Urinary tract infection is one of the most common infection in pregnancy .The urinary tract infection is often asymptomatic in pregnant women's. It was observed that significant proportion of pregnant women's had significant asymptomatic bacteriuria.The isolates showed huge percentage of ESBL Producers .which was alarming. Our study focus on need of careful screening antenatal women's for asymptomatic bacteriuria and also emphasizes the need for microbiology laboratories to adequately screen for ESBL-producing strains of the family Enterobacteriaceae, to avoid post partum complications in mother as well as new born.

Keywords: *Asymptomatic bacteriuria (ASB)*, Extended Spectrum Beta-Lactamase(ESBLs) Uropathogen, Pregnancy.

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Introduction:

Urinary tract infection (UTI) is one of the important bacterial infection occurred in pregnancy which may involve the lower urinary tract or the bladder.¹ Urinary tract infection (UTI) has been reported among 20% of the pregnant women.² The chances of acquiring bacteriuria vary during the course of pregnancy and it ranges from 0.8% in the early weeks to 1.93% towards the end of pregnancy.³ The physiologic changes like ureteral dilatation, increased bladder volume and decreased bladder tone, along with decreased ureteral tone occurring in the urinary tract due to pregnancy result in increased urinary stasis and ureterovesical reflux has a significant impact on the natural history of UTI during gestation. That's why UTI is common during pregnancy.⁴

UTI may be symptomatic or asymptomatic. The symptomatic forms may present as urethritis, cystitis, acute pyelonephritis and pyelonephritis with bacteremia or sepsis. The asymptomatic form known as asymptomatic bacteriuria (ASB) is defined as the presence of significant bacteriuria i.e., 10^5 organism per milliliter (ml), without the symptoms of an acute urinary tract infection. According to different studies carried out various health setups the prevalence of ASB in pregnancy is varies from 2.6% to as high as 45.3%.⁵

UTI in pregnancy if left untreated it will result into bad outcome of pregnancy, increases morbidity and mortality in infants due to neonatal sepsis, intra uterine growth retardation (IUGR) etc.

Antimicrobial resistance is considered to be the most serious health threats especially for the common infections like sepsis, diarrhea, pneumonia, urinary tract infections, gonorrhoea, malaria, tuberculosis, HIV, influenza.⁶

Antibiotic resistance among urinary tract pathogens has been known to be increasing worldwide especially in commonly used antimicrobial agents. More than 50% of *Escherichia coli* strains causing urinary tract infections are reported worldwide to be resistant to fluoroquinolones.⁷

Antimicrobial resistance has been identified as a major threat by the World Health Organization due to the lack of new antibiotics in the development pipeline and infections caused by multi-drug resistant pathogens becoming untreatable.^{8,9}

It is found in every class of antimicrobial agent though their resistance mechanisms vary among different groups or classes of antimicrobial agents. The predominant mechanism for resistance to the β -lactam antibiotics in gram-negative bacteria is the production of β -lactamase. The production of extended-spectrum β -lactamases (ESBLs) is an important mechanism which is responsible for the resistance to the third-generation cephalosporins. During the last two decades, ESBL producing

gram-negative bacilli have emerged as a major problem in many settings. The overall reported incidence of ESBL in GNB ranges from 6 to 87% in India.¹⁰ ESBL production should be tested by the conventional methods and should be reported along with routine antibiotic susceptibility testing in every microbiology lab, to help the physicians choose the appropriate antibiotics.¹¹

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae carry a broad-spectrum beta-lactamase enzyme that enables them to become resistant to a wide variety cephalosporin antibiotic & also to penicillin .¹² Emergence of antimicrobial resistance in uropathogens has become a matter of great public health concern. So this study is carried out to evaluate antimicrobial resistance pattern among commonly used antimicrobial agents against different isolates causing urinary tract infection in pregnancy. This study is carried out to identify number of ESBL producing strains among isolated uro-pathogens by using phenotypic methods.

Materials and Methods:

This Prospective study was conducted in the Department of Microbiology, B.K.L. Walawalkar Rural Medical College, Sawarde, Dist- Ratnagiri, Maharashtra. Pregnant women in different stages of pregnancy without symptoms of urinary tract infection attending the antenatal clinic of Obstetrics and Gynaecology were screened for significant bacteriuria, by quantitative standard loop culture method on 5% sheep blood agar and Mac-Conkey Agar.

The recovered isolates which fit into inclusion criteria are first identified by using standard bacteriological techniques i.e using different biochemical reactions as per departmental standard operating procedure for identification of gram negative, gram positive organisms. These different recovered isolates are also subjected to antimicrobial sensitivity testing to commonly use urinary antimicrobials. The isolates showing decreased susceptibility to 3rd generation cephalosporin's were subjected to detection of ESBL production by the standard disc diffusion testing method/ disc potentiation test recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines.¹³

Study type: Prospective study

Study duration: June 2018 to December 2018

Inclusion criteria:

Single organism grown with colony count $> 10^5$ org/ml (significant bacteriuria) were only subjected for further study. Exception to this is presence of growth morphologically suggestive of *Staphylococcus aureus*.

Exclusion criteria:

All cases having clinical complaints like fever, dysuria, burning sensation & incomplete voiding (symptomatic cases) indicating Urinary tract Infection, Pregnant women with known congenital anomalies of Urinary tract & other renal disorders, those were have history suggestive of taken antimicrobial agents recently. Pregnant women with active bleeding per vagina& those were in active labour were also excluded from study.

Gram positive isolates & insignificant growth of gram negative bacilli were not subjected to antimicrobial susceptibility and ESBL detection.

Antimicrobial Susceptibility Testing

The urinary pathogens isolated from these patients were tested for antimicrobial susceptibility towards following drugs using Kirby Bauer disc diffusion method on Mueller Hinton agar. (source of antimicrobial discs: Hi Media, India)

1. Ampicillin (10 µg)
2. Amoxicillin-clavulanic acid (20/10 µg)
3. Cefazolin (30 µg)
4. Cefuroxime (30 µg)
5. Cefotaxime (30 µg)
6. Ceftazidime (30 µg)
7. Nitrofurantoin (300 µg)
8. Fosfomicin (200 µg)
9. Cotrimoxazole (23.75/1.25 µg)
10. Norfloxacin (10 µg)
11. Gentamicin (10 µg)
12. Amikacin (30 µg)

ESBL detection is done as per CLSI guidelines by disc diffusion test using both cefotaxime and ceftazidime discs alone and in combination with clavulanic acid. Klebsiella pneumoniae ATCC 700603 (ESBL positive) and Escherichia coli ATCC 25922 (ESBL negative) strains used as controls in this study.

Material required

1. Isolates from urine sample received from pregnant women after informed consent.
2. Antimicrobial discs (Hi-Media, India) for ESBL detection –

- Ceftazidime 30 µg , Ceftazidime-clavulanic acid 30 µg/10 µg
 - Cefotaxime 30µg , Cefotaxime-clavulanic acid 30 µg/10 µg.
3. Muller Hinton Agar
 4. Control strains – *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) and *Escherichia coli* ATCC 25922 (ESBL negative).

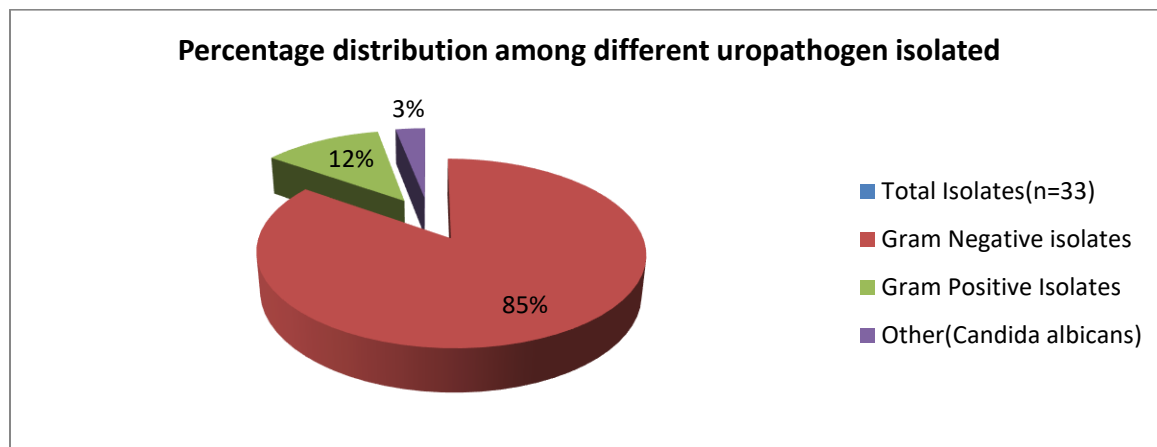
Result:

Out of 180 urine specimens examined from 180 pregnant women, 33 (18.33%) urine specimens showed significant growth. All of these females doesn't show any symptom related to UTI, indicating asymptomatic but significant bacteriuria cases. Among these isolates 85% isolates were gram negative organisms.(Figure No 1)The most common uropathogen isolated was *E. coli* (18 isolates), followed by *Klebsiella* species (8 isolates). Table 1 shows the distribution of different urinary pathogens isolated from these cases.

Table No.1: Different etiological agent isolated in pregnant women with asymptomatic bacteriuria

Organism isolated	No. of isolates
<i>E.coli</i>	18
<i>Klebsiella spp.</i>	8
<i>Staphylococcus aureus</i>	4
<i>Pseudomonas spp</i>	1
<i>Pseudomonas aeruginosa</i>	1
<i>Candida albicans</i>	1
Total	33

Figure No 1: percentage distribution amongst uropathogen

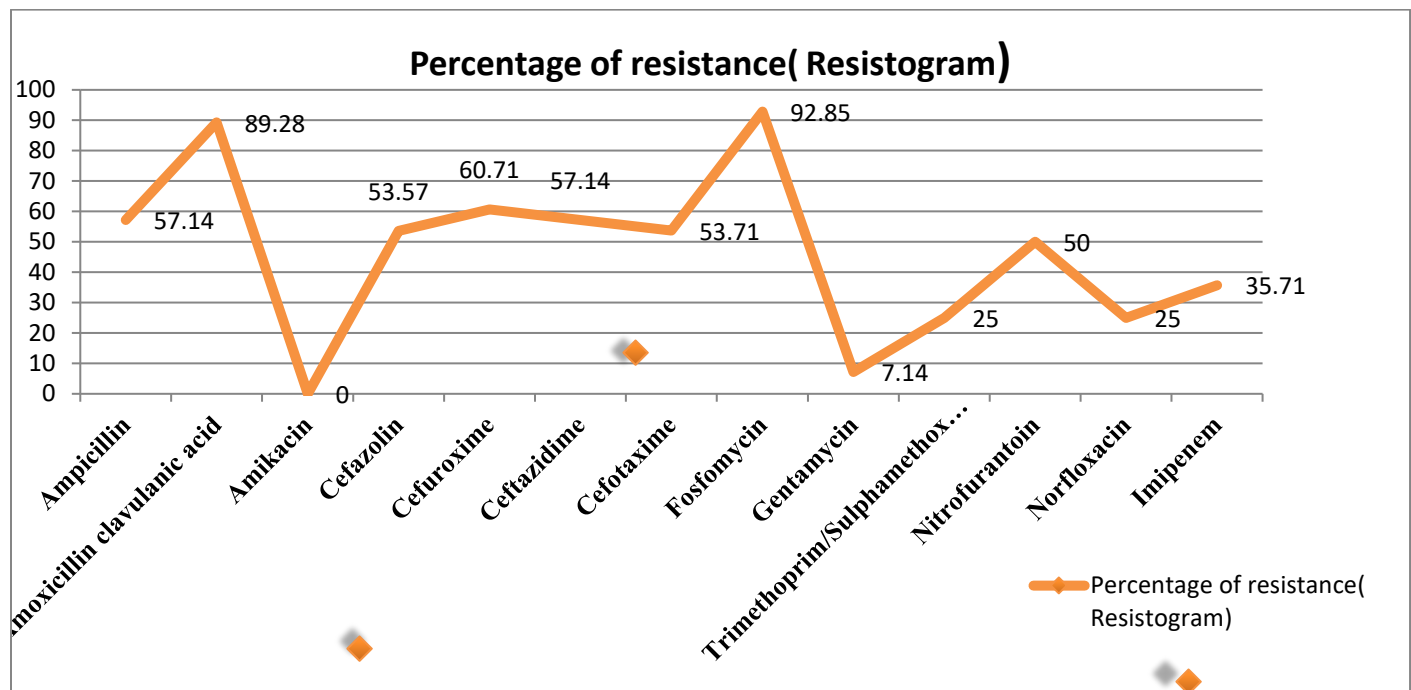


In our study isolated gram negative uropathogens were subjected to antimicrobial susceptibility by using Kirby bauer disc diffusion method. Bacterial uro-pathogens were revealed the presence of high levels antimicrobial resistances against commonly prescribed drugs as shown in Table 2. A significantly high resistance was noted to the, Fosfomycin (92.85%), Amoxicillin clavulanic acid (89.28%) & Cefuroxime (60.71%), Ceftazidime (57.14%), Cefotaxime (53.71%) & Nitrofurantoin (50%) by the Gram-negative isolates.(Figure No 2)

Table No. 2: Percentage Sensitivity Among Uropathogenis Isolated

GRAM NEGATIVE ORGANISMS	No of isolates	Ampicillin	Amoxicillin - clavulanic acid	Amikacin	Gentamycin	Fosfomycin	Cefuroxime	Ceftazidime	Cephataxime	Trimethoprim/Sulphamethoxazole	Nitrofurantoin	Norfloxacin	Cefazolin	Imipenem
<i>Escherichia coli</i>	18	60	11	100	88	6	39	39	44	78	50	72	66	66
<i>Klebsiella species</i>	8	8	0	100	100	12	50	37	37	60	25	88	50	62
<i>Pseudomonas species</i>	2	2	0	100	100	0	0	0	0	0	0	50	60	0

Figure No 2: Percentage resistance against commonly used antimicrobial agents in UTI



ESBL detection

Gram negative uropathogens were subjected to phenotypic ESBL detection method by using standard disc diffusion testing method/ disc potentiation test as per CLSI guidelines 2018.¹³ Among gram negative isolates 15 (54%) were ESBL producers. different isolates distribution of ESBL producers was observed in Escherichia Coli (10 out of 18), Klebsiella Species 62.5% (5in8) & Pseudomonas species (100%). (FIGURE 3, 4).

Figure No 3: ESBL Producing Strains VS Non ESBL Producing Strains Among Gram Negative Isolate (PERCENTAGE DISTRIBUTION)

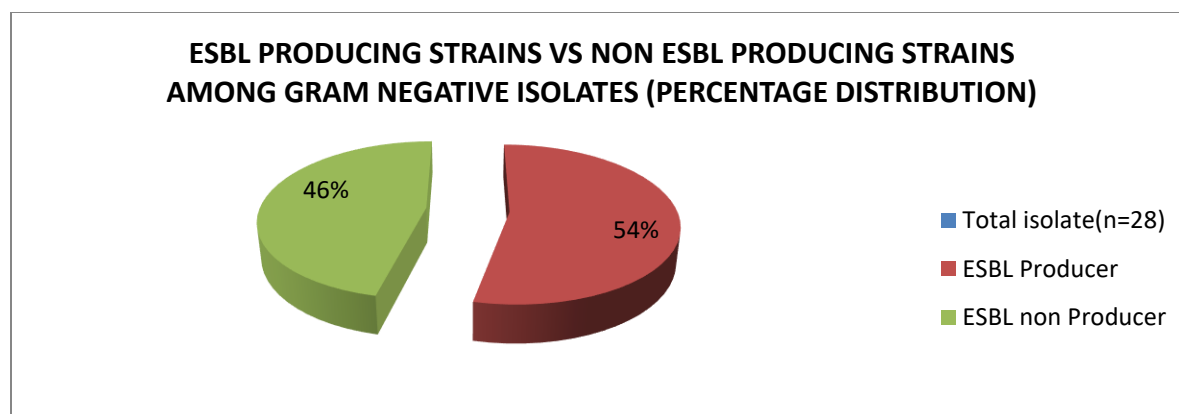
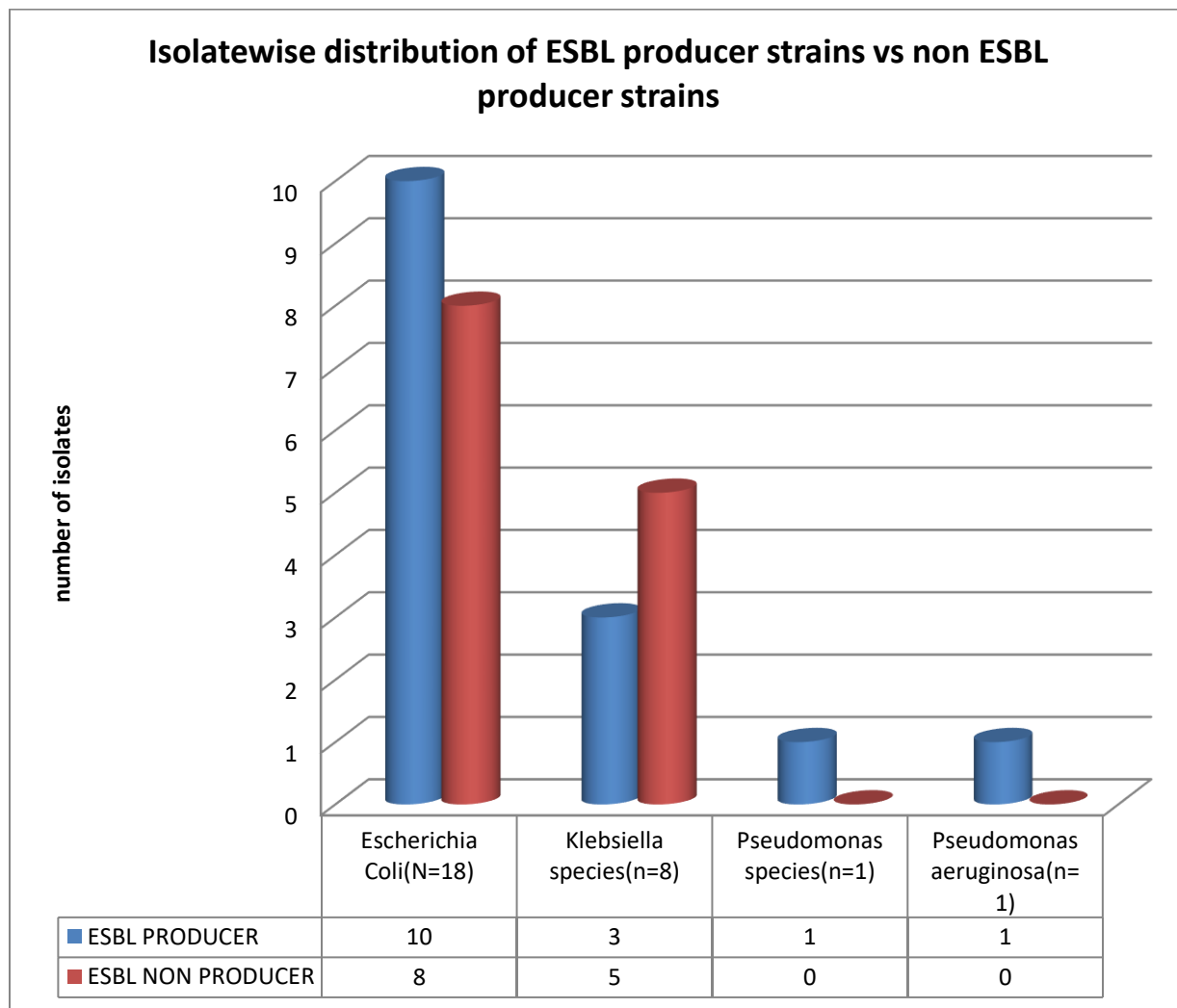


Figure4: Isolate wise Distribution of ESBL Producers



We also recorded antibiotic resistance profile of various ESBL and non ESBL strains against commonly used antimicrobial agents commonly used in UTI. In this study we observed that 3rd generation cephalosporin’s like Cefotaxime ,Ceftazidime showing high degree of resistance in ESBL producing strains compare to Non ESBL producing strains. But percentage resistance against Fosfomycin nearly similar.(Table 3)

Table No.3: Comparison of Resistogram in ESBL producing isolates & ESBL non Producers

Name of antibiotic	Resistogram in ESBLs (% Resistance)	Resistogram in NON ESBLs (%Resistance)
Ampicillin	66.66	46.15
Amoxicillin Clavulanic acid	89.28	92.3
Amikacin	0	0
Cefazolin	80	23.07
Cefuroxime	60	61.53
Ceftazidime	100	7.6
Cefotaxime	100	0
Fosfomycin	93.33	92.3
Gentamycin	6.6	7.6
Trimethoprim/ Sulphamethoxazole	33.33	15.38
Nitrofurantoin	60	38.46
Norfloxacin	33.33	15.38
Imipenem	40	30.76

Discussion:

Asymptomatic Bacteriuria (ASB) is very frequently observed among the pregnant woman and may lead to complications such as low birth weight, stillbirth, anemia and sepsis if left untreated. This necessitates the need to treat ASB at the earliest.

Screening for and treatment of ASB in pregnancy has become a standard of obstetric care and some antenatal guidelines include routine screening for ASB like WHO recommendations on antenatal care.¹⁴

The present study was conducted to evaluate the presence of asymptomatic *bacteriuria* in pregnancy and to observe pattern of drug resistance against commonly used antimicrobial agents

which were used for the treatment of the pregnant women's attending ANC Clinics at our rural tertiary care hospital in konkan region.

In this study antimicrobial susceptibility testing of these isolates was carried out to find out number of ESBL producing uropathogens among the recovered isolates. Only gram negative isolates were included for detecting antimicrobial susceptibilities and ESBL Producing ability of them. We have excluded all insignificant growth and gram positive isolates from study.

In this study, out of 180 urine specimens examined from 180 pregnant women, 33 (18.33%) urine specimens showed significant growth of uropathogenic organisms indicating asymptomatic UTI. This indicate in present study percentage of culture proven cases of UTI among asymptomatic pregnant females were 18.33%. Which fall between range of percentage of culture proven cases of asymptomatic bacteriuria (4% to 23.9%) observed in different studies conducted by various author.^{15,16,17}

Out of these 33 isolates 28 isolates were gram negative bacilli among these gram negative isolates ESBL producing isolates were 15 (53.57%) this was much higher compare to similar study carried out by Rekha Thapa et al in Nepal (7.30% of ESBL producers observed).¹⁸The incidence of ESBLs 19.8% was recorded in one study carried by Kumar M S et al & some studies carried out India also reported high incidence(60-68%) of ESBL producing gram negative bacilli.^{19,20,21}

In this study we found ESBL producing strains 10 out of 18 (55%) among E. coli isolates and 3 out of 5 (37.5%) the Klebsiella spp. Isolates. These results are slightly higher compared with one study which was carried out in the Department of Microbiology, Jhalawar Medical College and Hospital, Jhalawar, Rajasthan, India.²²

Conclusion:

The present study shows significant proportion of pregnant women had asymptomatic bacteriuria with alarming percentage of ESBL producing strains amongst bacterial isolates. As per available records this was first effort to analyze presence of ESBLs in *Asymptomatic bacteriuria* cases in pregnancy in a Konkan region.

The study emphasizes the need adequate screen for ESBL-producing strains amongst isolated organisms from asymptomatic bacteriuria cases because infections caused by ESBL producing strains are difficult to treat by using routinely used antimicrobial agents. Regular monitoring is required to establish reliable information about resistance pattern of uropathogens for optimal empirical therapy of patients with UTI.

One should avoid unnecessary antibiotics and optimize their use to minimize resistance pressure. The duration of the study was only 6 months. Further studies are recommended to include both symptomatic and asymptomatic pregnant women with UTI over a longer duration to establish etiology and a trend in antibiotic resistance pattern in pregnant women's coming from the catchment area covered by, tertiary care institute located in Ratnagiri district.

Limitations:

Shorter Study duration & less number of isolates included in study is major obstacle to generalized study results.

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