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## Original Research Article

## Detection of ESBL producing *E.coli* and *Klebsiella* spp in urinary tract infection due to long exposure of catheterize condition in Covid positive patient admitted in HDU

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## ABSTRACT

**Introduction:** Long exposure of broad spectrum antibiotic can severe causes of extended-spectrum beta-lactamase (ESBL) production within bacteria in UTI patient. Community-acquired infections due to ESBL producing *Escherichia coli* and *Klebsiellae* both are rising worldwide.

**Objective:** To assess the prevalence and antibiotics resistance patterns of ESBL-producing *Escherichia coli* and *Klebsiella* spp. from covid positive patient admitted in HDU with catheterized condition.

**Materials and Methods:** A hospital-based cross-sectional study was conducted on Salboni Super specialty Hospital (Covid Level-IV) at West Bengal, India from August 2020 to August 2021 and a total of 1116 urine samples were collected from catheterized patient admitted in male and female HDU due to exposure of covid. Samples were cultured on CLED agar and then Identification of the isolates was done by Gram's staining followed by biochemical tests. Antibiotic susceptibility testing was done by modified Kirby-Bauer disc diffusion method and interpretation was done following Clinical and Laboratory Standard Institute (CLSI) guidelines for detection of ESBL producing *E.coli* and *Klebsiellae* spp.

**Result:** present study showed 51.6% (n-326) *E.Coli* and 37.2% (n-235) *Klebsiella* spp. from 631 significant growths from both male and female patients. Out of this, total ESBL producing *E.coli* and *klebsiella* spp. seen 55% (n-309) from both male and female patient in different age group. Finally Isolated ESBL producing *E.coli* 45.9 % (n-142) and ESBL producing *Klebsiella* spp. 54 % (n- 167) from both male and female HDU.

**Conclusion:** Detection of ESBL producing *E.coli* and *Klebsiellae* due to prolonged uses of broad spectrum antibiotic vigorously. The occurrence of multidrug resistance to the third-generation cephalosporins, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, and tetracyclines is more common among ESBL producers. It may help to detect other organism in same condition, so that it will be importance in clinical aspect of future treatment for clinician.

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## 1. Introduction

Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and *Klebsiellae* have been recognized as a major

multidrug-resistant bacteria implicated in serious hospital and community-acquired infections worldwide, especially in places where poor sanitation, and inadequate hygienic practices are very common.<sup>1–4</sup> antimicrobial resistance (AMR) is considered a main global threat to human and animal health<sup>5</sup> AMR is responsible for thousands of human Fatalities annually.<sup>6</sup> In the last decade, the global growth of livestock has been associated with an increase in antibiotics use.<sup>5</sup> Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) represent one of the highest burdens of AMR to public health and have globally spread in both hospital settings and the community.<sup>7</sup> Extended-spectrum  $\beta$ -lactamases (ESBLs) are class A  $\beta$ -lactamases, a rapidly evolving group of  $\beta$ -lactamases with the ability to hydrolyze and cause resistance to the oxy-imino cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime) and monobactams (aztreonam).<sup>8</sup>

ESBL-producing *E. coli* and *K. pneumoniae* are the predominant cause of childhood infections and present significant challenges<sup>9</sup> such as development of adverse outcomes.,<sup>10</sup> treatment failure due to multidrug resistance, and high morbidity and mortality.<sup>11</sup> Empirical and symptomatic (without a diagnosis) use of antibiotics in resource poor settings is responsible for higher incidence of antibiotic resistance among bacteria.<sup>12</sup>

ESBLs are often encoded by genes located on large plasmids, and these also carry genes for resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol.<sup>13</sup> Recent studies have demonstrated fluoroquinolones resistance mediated by co-transfer of the *qnr* determinant on ESBL-producing plasmids.<sup>14,15</sup>

Thus, very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing enterobacterial isolates. As a result, ESBL-producing organisms pose a major problem for clinical therapeutics. This review attempts to present a comprehensive picture on the basis of the currently available literature about this diverse, complex and rapidly evolving group of enzymes.

## 2. Materials and Methods

The current study was carried out in the department of pathology, salboni super speciality hospital, WB, India from August 2020 to August2021 among catheterize patient admitted in ICU for covid purpose. The urine sample were collected from patient in sterile container and cultured on CLED (cystine Lactose Electrolite Deficient) agar, after bacterial growth we further proceed for detection of *Escherichia Coli* and *Klebsiella* spp. through biochemical like Indole, MR,VP, Citrate, Urease, TSI and Mnitol tests.

Antibiotic test was done by the Kirby Bauer Disc diffusion method on Muller Hinton agar. Antimicrobial

agents tested were Ampicillin, Amoxiclav, gentamicin, amikacin, nalidixic acid, norfloxacin, ofloxacin, nitrofurantoin, sulphofurazole, cotrimoxazole, piperacillin-tazobactam, cephalothin, cefixime, cefuroxime, ceftriaxone, ceftazidime, Cefepime, cefotaxime, imipenam, meropenemrecommended by the CLSI 2013 using *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 700603 as a standard strain for routine quality control.

### 2.1. Phenotyping detection

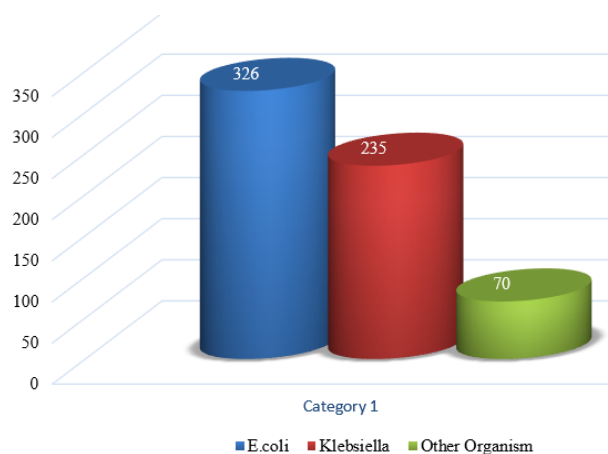
A phenotypic confirmatory test recommended by the CLSI was done by combined disc diffusion method. ESBLs was detected by the confirmatory method of Clinical and Laboratory Standards Institute (CLSI) using ceftazidime (30 mg) and a disc of ceftazidime plus clavulanic acid (30/10 mg) were placed at a distance of 20 mm on a lawn culture (0.5 McFarland inoculum size) of suspected ESBL producing clinical isolate on Mueller-Hinton Agar (MHA, Hi-Media, Mumbai). *Escherichia coli* ATCC 25922 were used as the negative control and *Klebsiella pneumonia* ATCC 700603 were used as the ESBL positive control. The study was approved by ethics committee of Salboni SSH,WB, India.

### 2.2. Statistically analysis

Results were analyzed using Pearson's chi-square test, or Fisher's exact test. Avalue of  $P < 0.05$  was regarded as statistically not significant (SPSS 16.0, SPSS Inc., Chicago, IL, USA).

## 3. Result

Total 1116 catheterize urine sample were collected from Male and Female HDU patients during one year of duration (August 2020 to August2021) in covid pandemic situation.



**Fig. 1:** Analysis of different isolated organism from bacterial growth

**Table 1:** Statistical analysis of bacterial growth

Age group	Female HDU patient		Male HDU patient	
	Growth	Non-Growth	Growth	Non-Growth
Years	Numbers(N) with Percentage%	Numbers(N) with Percentage%	Numbers(N) with Percentage%	Numbers(N) with Percentage%
30-39	110(52%)	99(48%)	102 (53%)	89 (47%)
40-49	119(54%)	100(46%)	100 (52%)	91 (48%)
50-59	100(65%)	52(35%)	100 (64%)	54 (36%)
P-value	7.0226,P=0.0298,P<0.05 Significant		7.4182, P=0.0244,P<0.05 Significant	

**Table 2:** Analysis of different isolated organism from bacterial growth

Age Group	<i>E.coli</i> (326)		<i>Klebsiella</i> spp. (235)		P value
	Numbers(n)	Percentage (%)	Numbers(n)	Percentage (%)	
Years					6.1666
30-39	115	54%	95	44%	P=0.0458
40-49	111	51%	90	41%	P<0.05
50-59	100	50%	50	25%	Significant

**Table 3:** Statistically analysis of ESBL production from male and female patients

Age group	ESBL (309)		Non-ESBL (252)		P value
	Numbers(n)	Percentage (%)	Numbers(n)	Percentage (%)	
Years					0.9522 P=0.62121
30-39	105	50%	92	43%	P<0.05
40-49	99	49%	84	39%	Not-Significant
50-59	105	70%	76	30 %	

**Table 4:** ESBL producing *E.coli* and *Klebsiella* spp. in between male and female

Age group	ESBL Producing <i>E.Coli</i> (142)		ESBL Producing <i>Klebsiella</i> (167)		P-Value
	Numbers(n)	Percentage (%)	Numbers(n)	Percentage (%)	
30-39	63(44.3%)		56 (33.5%)		3.9354 P=0.139775 P<0.05 Not-Significant
40-49	42(29.5%)		62 (37.1%)		
50-59	37 (26%)		49 (29.3%)		

Studied in three different age group (30-39, 40-49, and 50-59) in between male and female (Table 1). In present study showed significant bacterial growth (n=329, P=0.0298) in female and in male (n=302, P=0.0244). After identification of bacterial, growth showed (n=326) 51% *E.coli* growth and (n=235) 37% *Klebsiella* growth and (n=70) 11% other organism causing urinary tract infection. Furthermore, total ESBL producing *E.coli* and *Klebsiella* spp. seen (n=309) 55% (P=0.62121) in between male and female patient. After that finally ESBL producing *E.coli* seen 45% (n=142) and ESBL producing *Klebsiella* Spp. seen 54% (n=167) in respect to age group (30-39, 40-49, and 50-59 in both male and female HDU patient.

#### 4. Discussion

In this study the first description of ESBL producing uropathogenic *E. coli* and *Klebsiella* spp. involved in major public health cases of urinary tract infections in our country. Urinary tract infections are the most common infections in adults' patients and *E. coli* and *Klebsiella* being leading pathogenic agent in these infections; it was our matter of interest is to excess ESBL Producing *E.coli* in catheterized

patient admitted in HDU in covid situation.

Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens and prevalence varies with the age and sex. In this present study, it was noted that the significant bacteriuria was found in high prevalence (P-value = 0.0298) of UTI positive clinical sample in male and female in the age groups of 40-49 years. Similarly, study was observed Parveen RM<sup>16</sup> et al, 2011 in Puducherry, India and 39 isolates investigated, 37 (94.8%) were found to be resistant to at least one of the third generation cephalosporins. Among these 37 isolates, 36 (97.2%) were found to be ESBL positive by phenotypic testing from blood. And others study was observed Shakya Pet al,<sup>17</sup> 2017 in Nepal and showed significant bacteriuria with 365 (80.9%) *E. coli*, 17 (3.8%) *Klebsiella pneumoniae* and 3 (0.7%) *Klebsiella oxytoca*.

Concurrently, significant (P=0.0458) growth of *E.coli* and *Klebsiella* spp. was seen for both male and female patient, similarly study was observed on Kayastha et al,<sup>18</sup> 2020 and showed *E. coli* (n = 79), *Klebsiella pneumoniae* (n = 18), and *Klebsiella oxytoca* (n = 6) were isolated from different clinical specimens.

We showed non-significant of ESBL producing *E.coli* and *Klebsiella* ( $P=0.139775$ ) from both male and female (Table 4) patient dividing age group of 30-39, 40-49, and 50-59, similarly the study was done by Abayneh M et al,<sup>19</sup> 2018 in Canada and study showed ESBL-producing phenotypes were detected in 23% (n - 17) of urinary isolates, of which *Escherichia coli* accounts for 76.5% (n - 13) and *K. pneumoniae* for 23.5% (n - 14).

## 5. Conclusion

Use of broad-spectrum antibiotic during long period can cause of multiple drug resistant (MDR) *E.coli* and *Klebsiellae* spp. detected catheterize urine sample in covid patients.

The present study concludes that, high prevalence of *E. coli* and *Klebsiella* associated with UTI and illustrates the maximum number of organisms from female patients of catheterize condition in age group i.e. 25-44 years. Most of the *E. coli* and *Klebsiella* isolates showed multi drug resistance. The most effective drug was found to be Nitrofurantoin followed by Amikacin, Gentamicin and Imipenem. Amikacin, Gentamicin and Imipenem are the parental drugs but only Nitrofurantoin which showed good activity is an oral drug. So, Nitrofurantoin remains the only available alternative with high susceptibility rate among oral agents for UTI. To provide optimum use and to avoid misuse and over use of these drug, culture and susceptibility testing is the need of hour to preserve it for next generation.

Hence, forth concluded that microbiological diagnosis is very essential for better treatment so that appropriate antibacterial drug can be initiated on time in order to limit the worsening of condition and to prevent complications and should not take antimicrobial drugs vigorously without clinician advise.

## 6. Conflict of Interest

None.

## 7. Source of Funding

None.

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