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Original Research Article

Phenotypic detection of extended spectrum beta lactamase and metallo beta lactamase producers among multidrug resistant *Escherichia coli* and *Klebsiella* spp. in urinary tract infections

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ABSTRACT

Introduction: The incidence of Urinary tract infection (UTI) concomitantly causing the morbidity and mortality in patients with specific risk factors is highly alarming. *Escherichia coli* (*E. coli*) and *Klebsiella* spp., are the most frequently isolated species and considered as highly significant due to their ability to produce Extended spectrum beta lactamase (ESBL) and Metallo beta lactamase (MBL). UTIs caused by bacteria that produces ESBL and MBL are becoming more common, and the ability of diagnostic microbiology laboratories to promptly screen for and identify these resistant infections is crucial.
Objectives: The main objective of my study is to identify and its susceptibility pattern of ESBL and MBL producing *E. coli* and *Klebsiella* spp., causing UTI.
Materials and Methods: A total 0f 200 multi drug resistant (MDR) *E. coli* and *klebsiella* spp., were screened for ESBL as well as MBL production by phenotypic methods.
Results: From a total of 350 significant UTI cases, 135 are *E. coli* and 65 are *Klebsiella* spp., remaining are comprised of other bacteria such as Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis. Among 135 *E. coli* isolates 56 found to be multidrug resistant and 42 were ESBL producers and 9 were MBL producers. Among 65 *Klebsiella* spp., isolates 23 were multidrug resistant and 22 were ESBL producers and 6 were MBL producers.
Conclusion: This study reveals the prevalence of ESBL and MBL producing multidrug resistant *E. coli* and *Klebsiella* spp., in urinary tract infections as well as their significant role in treatment failure.

1. Introduction

The incidence of urinary tract infection (UTI) concomitantly causing the morbidity and mortality in patients with specific risk factors is highly alarming. Women are significantly more likely to get UTI than men due to inherent reasons.[1] A large proportion of these infections are caused by nosocomial pathogens often leading to treatment failure. The most commonly isolated species are *E. coli* and *Klebsiella* spp., and these are considered as highly significant due to their ability to produce Extended spectrum beta-lactamase (ESBL) and Metallo Beta-Lactamases (MBL), therefore, selection of appropriate antibiotic therapy is difficult.[2] As per the data of the World Health Organization (WHO), the mortality rate due to infection with MDR organisms is significantly much greater than that of non-MDR organisms.[3] Carbapenems

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are considered as reserve class of antibiotics used to treat life threatening Gram-negative bacterial infections. However, their indiscriminate use worldwide resulted in the emergence of resistance to these agents. Of the several mechanisms responsible for their resistance; production of ESBLs and MBLs play vital role often complicating the clinical management of the patient.[4] For the identification and categorization of ESBL and MBL, Polymerase chain reaction (PCR) based genotyping techniques continue to be the gold standard; nevertheless, their application is primarily limited to research.[5] As a quick way to find MBL activity, most of the diagnostic labs still use phenotypic testing based on culture. Phenotypic methods offer advantages of being economical, easy to perform on routine basis with adequate sensitivity. Early identification of organisms that produce ESBL and MBL is important because it enables the timely administration of the right medications to successfully manage illness.[6–11] Determining the frequency of ESBL and MBL producing *Escherichia coli* and *Klebsiella* spp., by phenotypic approaches is the purpose of the current study.

2. Materials and Methods

At the Department of Microbiology, SVS Medical College and Hospital, Mahbubnagar, this prospective study was carried out from September 2017 to July 2019. Institutional ethics committee approval (SVS Medical College/Institutional Ethical Committee; approval no.: ECR/704/Inst/AP/2015/RR-18) was duly obtained prior to the study initiation. Midstream urine samples received in the microbiology clinical laboratory from the patients (IP and OP) having symptoms suggestive of UTI were processed appropriately for bacterial culture and sensitivity. Samples were cultured on Blood agar and MacConkey agar and incubated at 37°C for 18-24 hours. All patient samples with significant ($\geq 10^5$CFU/ml) growth of *E. coli* and *Klebsiella* spp., were included in the study. Samples from age group of below 12yrs and from patients treated with antibiotics prior to urine culture were excluded from the study. Pure isolates were characterized by Gram stain and biochemical reactions like indole test, methyl red test, Voges- prausker test, citrate test, urease test, triple sugar iron test etc. and were confirmed by VITEK 2 compact system (biomerieux, France). Excel format is used for data entry and the software version 6 of graph pad prism and SPSS version 20 were utilized for statistical analysis, respectively.

2.1. Test for antibiotic susceptibility

In compliance with the 2017 CLSI standards, the Kirby Bauer disc diffusion method was used to test for antimicrobial susceptibility. Antibiotics discs (Himedia) used were Amikacin (30μgm), Piperacillin-tazobactam (10μg), Ampicillin (30μg), Cefotaxime (30μg), Ceftazidime (30μg), Ceftriaxone (30μg), Cefpodoxime (30μg), Nitrofurantoin (30μg), Trimethoprim-sulphamethoxazole (1.25+23.75μg), Ciprofloxacin (5μg), Ofloxacin (5μg), Imipenem (10μg), Meropenem (10μg). Multidrug resistant bacteria were those that shown resistance to three or more distinct classes of antibiotics.[12] The CLSI guidelines state that any isolate with a zone size of less than 22 mm for ceftazidime, less than 27 mm for cefotaxime, less than 25 mm for ceftriaxone, and less than 17 mm for cefpodoxime has the possibility of harbouring pathogens that have the potential to produce ESBL and these isolates were taken for identifying ESBL production by phenotypic methods. Positive and negative controls, *Klebsiella pneumoniae* ATCC 700603 and *Escherichia Coli* ATCC 25922 were utilized in our study.

2.2. Confirmation of ESBL producers

ESBL production was further validated by combined disc assay for the isolates that the screening test had shown were ESBL positive. In this method, a disc of Ceftazidime (30μg) and Cefotaxime (30μg) along with Ceftazidime/clavulanic acid (30/10μgm) and Cefotaxime/clavulanic acid discs(30/10μgm) respectively were placed at a distance of 25mm on lawn culture of test organisms on Mueller Hinton agar plate (MHA) and incubated for 18-24hrs at 37°C. It was determined to be ESBL positive when the zone diameter of at least $\geq$ 5mm was more with Ceftazidime/clavulanic and Cefotaxime/clavulanic acid discs when compared to Ceftazidime and Cefotaxime alone.

2.3. Detection of MBL producers

Combined disc assay (CDT) using Imipenem and Imipenem/ethylenediaminetetracetate discs (EDTA) was used to confirm the MBL production in *E. coli* and *Klebsiella* spp., which are resistant to carbapenem drugs (Imipenem, Meropenem). Imipenem-ethylenediaminetetracetate (EDTA) disc was shown to have an inhibition zone that was $\geq$7mm greater than that of the imipenem disc alone, indicating MBL positivity.

3. Results

A total of 2520 urine samples were screened for bacterial culture during the period of September 2017 to July 2019 from the patients attending SVS medical college and hospital. About 350(12%) samples showed significant bacteriuria.

The prevalence of significant bacteriuria was observed at a high frequency between the age group of 21-30yrs age (31.45%) followed 61-70 years (24%) and low among 51-60 years age (7.4%) as shown in Table 1. In all age groups, the frequency of occurrences among women was higher than that of men, with a ratio of 2.3:1.

In 350 UTI cases 200 (57.2%) are *E. coli* and *Klebsiella* spp., and 150(42.8%), are the other bacteria including both Gram-positive *cocci* and various Gram-negative bacilli which are largely responsible for the remaining cases. Among 200 isolates *E. coli* was the most common bacteria isolated with a frequency of 135(38.6%) followed by *Klebsiella* spp., in 65 (18.6%), details as shown in Figure 1.

3.1. E.coli and Klebsiella spp , antibiotic resistance pattern

The antibiotics resistance pattern of the *E. coli* and *Klebsiella* spp., were displayed in Table 2. *E. coli* had shown the lowest rate of resistance towards Nitrofurantoin (6%) followed by Imipenem (25.1%) and highest rate of resistance towards Ceftriaxone (71.1%). *Klebsiell*a spp., had shown the lowest rate of resistance towards Nalidixic acid (10.7%) followed by Imipenem (27.6%) and highest rate of resistance seen against Ceftriaxone (60%).

3.2. The incidence of ESBL production in E. coli and Klebsiella spp

Based on their resistance pattern, 56 of the 135 *E. coli* isolates were identified as MDR strains; 42 (31.1%) of these isolates were found to be positive for ESBL production. On the other hand, out of 65 *Klebsiella* spp., 23 were identified as MDR isolates and among them 22 (33.8%) were found to produce ESBL (Figure 2A and B).

3.3. The incidence of MBL production in E. coli and Klebsiella spp.,

carbapenem resistance screening was performed on all 135 *E. Coli* samples. It showed that only a small percentage 24 (17.7%) are resistant to carbapenem**.** Among them 9 (6.6%) isolates were positive for MBL production.

However, out of 65 *Klebsiella* spp., 19 (29.2%) carbapenem resistance were identified and among them 6 (9.2%) were positive for MBL production. Details as shown in Figure 3A and B.

4. Discussion

Urinary tract infections are the most prevalent type of bacterial infection both in the community setting and in the hospital setting. The purpose of this study was to evaluate the magnitude of antibiotic resistance exhibited by *E. Coli* and *Klebsiella* spp., to commonly prescribed drugs, as well as the frequency of ESBL and MBL production among these

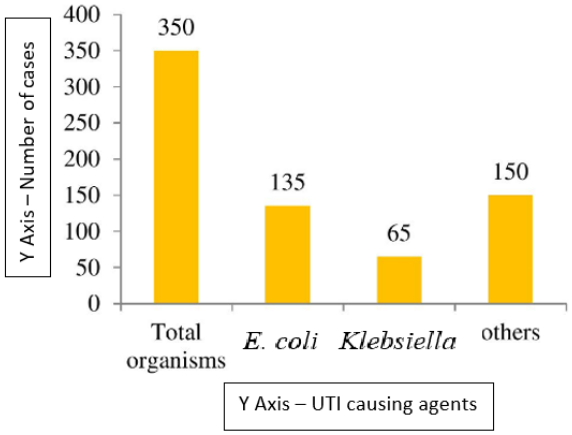

Figure 1: Incidence of *E. coli*, *Klebsiella* sps and other bacteria

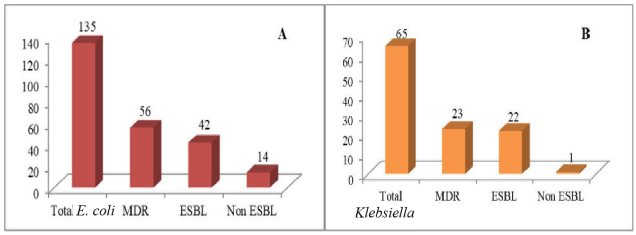

Figure 2: a,b: Prevalence of ESBL production in *E. coli* and *Klebasiella* species

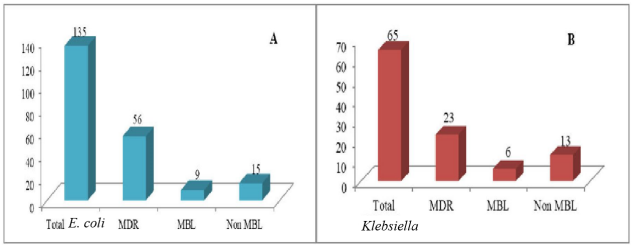

Figure 3: a,b: Prevalence of MBL production in *E. coli* and *Klebsiella* species

bacteria as a result of inappropriate and increased antibiotic use.[13]

In India, there are significant differences in the incidence of bacteria that produce ESBL and MBL between hospitals and even between different infection sites including wound infections, urinarytract infections and septicemia. Furthermore, most of the hospitals are clueless about antibiotic sensitivity patterns of most commonly occurring bacterial species. This could lead to the incorrect prescription of antibiotics, which could result in the development of multidrug resistant bacteria. The sensitivity pattern of uropathogens in the present study shows increased rate of sensitivity to Nalidixic acid, Imipenem

Table 1: Age wise distribution of significant bacteriuria

S.No.	Age group (yrs.)	Male	Female	No of patients	Percentage (%)
1	11-20	15	27	42	12%
2	21-30	10	100	110	31.4%
3	31-40	12	34	46	13.1%
4	41-50	15	27	42	12%
5	51-60	12	14	26	7.4%
6	61-70	44	40	84	24.1%
	Total	108	242	350	100%

Table 2: Antibiotic resistant pattern of *E. coli* and *Klebsiella* species

Antibiotics	*E.coli* (135)	Percentage (%)	*Klebsiella* (65)	Percentage (%)
Ampicillin	87	64.4	34	52.3
Amoxyclav	67	49.6	37	56.9
Piperacillin tazobactam	43	31.8	30	46.1
Ceftazidime	86	63.7	37	56.9
Ceftriaxone	96	71.1	39	60
Amikacin	46	34.07	31	47.6
Gentamicin	52	38.5	22	33.8
Ciprofloxacin	89	65.9	35	53.8
Norfloxacin	84	62.2	35	53.8
Ofloxacin	71	52.5	28	43.07
Nalidixic acid	48	35.5	7	10.7
Trimethoprim sulphamethoxazole	68	50.3	22	33.8
Imipenem	34	25.1	18	27.6
Nitrofurantoin	8	6	24	36.9

and Nitrofurantoin compared to remaining antibiotics. The lower sensitivity to Quinolones and third generation Cephalosporins is most likely due to their widespread empirical use in our region. In this situation, Nitrofurantoin appears to be the only promising oral antibiotic.[14]

In addition to the inherent anatomical differences, the possible reasons for higher frequency of UTI in young women as evidenced in this study, this could be due to complicated physiology particularly during the gestational period. Furthermore, several contraceptives can increase the risk of urinary tract infections.[15,16]

The current study shows that the patients in the group of 20-30 years had the increased frequency of UTI with significant bacterial growth followed by 60-70 years group and least in the age group of 50-60 years. Thakur et al.,(2013) observed Similar findings in their study done at the National Public Health Laboratory in Teku and Kathmandu, Nepal.[17]

As anticipated in general, *E. coli* was the most predominant bacteria isolated followed by *Klebsiella* spp., from community acquired UTI cases, the findings of this study agreed with others.[18–20] However, in patients on ventilators, catheters or following other surgical interventions were prone to get hospital acquired UTI involving predominantly *Klebsiella* spp., and other Gram positive and Gram-negative nosocomial pathogens.[21]

Antimicrobial resistance has recently been identified as a growing global issue, particularly in Gram negative bacteria.[22] β-lactam antibiotics are most preferred and often the mainstay in the treatment of UTI. Nevertheless, acquired resistance to these particular antibiotics in Urinary tract infection pathogens is frequently increased by bacterial enzymes, resulting in the formation of ESBLs.[23] In this current study antibiotics were utilized against 135 E. coli isolates, wherein Ceftriaxone, Ciprofloxacin, Ampicillin, Ceftazidime and Norfloxacin showed high resistance with 71.1%, 65.9%, 64.4%, 63.7% and 62.2% respectively. However, according to Perez et al.(2007), 94% of *E. coli* isolates are resistant to Ceftriaxone.[24] Ceftriaxone, Amoxyclav, Ceftazidime, Ciprofloxacin, Norfloxacin, and Ampicillin exhibited high resistance with 60%, 56.9%, 56.9%, 53.8%, 53.8%, and 52.3% against 65 *Klebsiella* spp., This disparity could be due to extensive usage of the pertinent antibiotic in that geographical area.

Increasing resistance to broad spectrum Cephalosporins have been identified in *E. coli* and *Klebsiella* spp., mostly as a result of the emergence of ESBLs in numerous countries.[25,26] We observed the prevalence of multidrug resistant with 41.4% of *E. coli* and 35.3% of *Klebsiella* spp., Various studies in Nepal have reported rates of Multidrug resistance ranging from 38.2 to 95.52% in *E. coli* and 25-100% in *Klebsiella* spp.,.[27] Prolonged hospitalization along with prior antibiotic usage are Common risk factors for

infection by multidrug resistant bacteria.[28]

In study we conducted, the prevalence of production of ESBL was 32%, with 31.1% of the *E.coli* and 33.8% of the *Klebsiella* spp., reporting ESBL positive. In a study conducted by Raut et al., the incidence of ESBL producing *E. coli* and *Klebsiella* spp., was reported to be as low as 18.2 and 4.1% respectively,[29] while it was as high as 80% for *E. coli*.[30] and 90.9% for *Klebsiella* spp.,.[31] The global incidence of ESBL production among clinical isolates ranges from < 1 to 74%.[32] Maximum ESBL production seen among *Klebsiellapneumoniae* (52.27%) followed by *E. coli* (46.43%).[33] *E. coli* (65.32%) and *Klebsiellapneumoniae* (24.9%) were the most common isolates in a study in Madhya Pradesh 2012, where 50.14% of the *E. coli* was ESBL producers.[34] *E. coli* is the major ESBL producing bacteria reported by paruji et al[35] and Khanfar et al.[25] All these studies were in agreement with finding from our study where *E. coli* and *Klebsiella* spp., were most frequent isolates and common producers of ESBL. In a study in Malappuram in 2016, maximum ESBL producers are obtained from urine samples.[36] According to Guragain et.al there is slight increase in ESBL producing bacteria in urinary isolates.[37]

Concerns have been raised about the Production of metallo-beta-lactamases (MBLs), particularly by members of the Enterobacteriaceae family.[38] A major risk to public health arises from the prevalence of MBL-producing bacteria in society because these organisms are resistant to carbapenems (Imipenem and Meropenem), which are antibiotics used to treat serious bacteria which produce ESBL.[39,40]

The prevalence of MBL strains vary markedly between different geographical regions within the country and from neighboring countries. Clinical isolates of *E. coli* and *Klebsiella* spp., currently exhibit an increasing incidence pattern of MBL production. Therefore, to track their incidence it is crucial to investigate their prevalence. According to our findings from our study, we reported, 6.6% of carbapenem resistant *E. coli* and 9.2% of *Klebsiella* spp., were MBL producers. According to the study conducted in Pakistan 71% of *E. col*i isolates was MBL producers,[41] which is alarmingly higher than our findings. In accordance to Indian study 7.03% E. coli isolates were MBL producers.[42] Research from various countries has revealed MBL production for *E. coli* ranging from 13.4 to 61.5% and for *Klebsiella* spp., it is from 33 to 36%.[43,44]

5. Conclusion

This is the first study from this region to reveal the incidence of Extended spectrum β lactamase and metallo β lactamase producing *Escherichia coli* and *Klebsiella* spp., from UTI and their significant contribution to the treatment failure and consequently increasing health care burden. Due to high prevalence of ESBL among these bacteria, the usage of carbapenems were on gradual rise which could be major cause of MBL mediated resistance. On the other hand, extensive usage of antibiotics and growth promoting agents in poultry, veterinary, agricultural practices and other non-clinical purposes might have also played an important role in the establishment and spread of MDR strains.[45] Therefore, proper guidelines and stringent vigilance for their usage and disposal in these non-clinical sectors also very essential to prevent drug resistance.

6. Conflict of Interest

The authors declare that they have no conflict of interest.

7. Source of Funding

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