www.wcprj.com 2025 Page: 116-125

World Current Pharmaceutical Research Journal

Research Article

Volume: 01
Issue: 04

DETECTION OF METALLO β-LACTAMASE AND EXTENDED SPECTRUM β-LACTAMASE PRODUCING BACTERIA IN URINE SAMPLES FROM URINARY TRACT INFECTED PATIENTS

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Article Received: 09 May 2025
Article Revised: 29 May 2025
Published on: 19 June 2025

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ABSTRACT

A total of 43 urine samples were examined for the presence of uropathogenic bacteria. Escherichia coli was isolated from 33 of these samples, while Klebsiella species were found in seven (7). Bacterial identification was confirmed using standard biochemical tests following CLSI guidelines. E. coli tested positive for Indole and Catalase, whereas Klebsiella spp. were negative for Indole and Oxidase; both organisms showed lactose fermentation on MacConkey agar. All confirmed isolates were screened for Metallo-β-lactamase (MBL) and Extended-Spectrum β-lactamase (ESBL) production. MBL activity was observed in two E. coli isolates using the Meropenem-EDTA Disk Synergy Test, indicated by a ≥7 mm increase in the inhibition zone. ESBL production was confirmed in one E. coli and three Klebsiella isolates using the Double Disc Diffusion Test with Ceftriaxone and Clavulanic Acid, where a zone enhancement of ≥8 mm signified ESBL activity. Three selected isolates underwent Sanger sequencing and were analyzed through BLAST. These results underscore the presence of multidrug-resistant pathogens in urinary tract infections and highlight the importance of ongoing antimicrobial resistance surveillance.

INTRODUCTION

The increasing prevalence of community- and hospital-acquired infections caused by multi-drug resistant (MDR) bacteria is a major concern. A key factor in this issue is the widespread and inappropriate use of antimicrobial drugs, which can lead to resistance in pathogenic bacteria.[1] Without a full understanding of the factors contributing to AMR and no concrete plans to address it, predictions suggest that by 2050, AMR will cause 10 million deaths annually.[2] AMR can spread through horizontal gene transfer between bacteria, with commensal bacteria playing a crucial role in this process. metallo β-lactamase (MBL) encoding genes are usually carried by mobile genetic elements that facilitate horizontal gene transfer (HGT) between bacteria and harbour a great ability to spread.[3,4]

The first acquired MBL (imipenemase; IMP-1), was reported from clinical isolates of *P. aeruginosa* and *Serratia marcescens* in Japan in the 1990s.[5] The important acquired MBLs, comprising the IMP, NDM, and VIM types, fall into subclass B1. They hydrolyze all currently available β-lactam antibiotics except monobactams (e.g., aztreonam)[6] They also further, which may also interfere with the interpretation of ESBL detection methods, since they also hydrolyze extended spectrum -lactams. Carbapenems evade most β-lactamases but are hydrolyzed by MBLs as well as by a few active-site serine β-lactamases (SBLs), notably members of the (carbapenemases) KPC and OXA-48-like groups.

The continuous monitoring of drug resistance in clinical isolates is essential to assess the true extent of the issue and to develop effective policies aimed at reducing drug resistance in bacteria. Additionally, understanding local antimicrobial susceptibility patterns is crucial for initiating timely and appropriate initial treatment. The varied structural characteristics of MBLs such as low sequence similarity, the presence of a metal ion at the core and shallow active site groove enable these enzymes to break down most beta-lactams while also being resistant to all known beta-lactamase inhibitors. Therefore, this study aimed to assess the prevalence of ESBL and MBL production in *E. coli* and *Klebsiella sps.*, isolated from clinical samples, along with their antimicrobial susceptibility profiles.

MATERIALS AND METHODS

Study Population

Fifty two patients were recruited in the study from OPD of Department of Urology, KLE hospital, Belagavi from September, 2024 to January 2025.

The patients with confirmed cases of urinary tract infection were enrolled in the study prior to initiation of antibiotic treatment. Patients with diabetes and other chronic diseases were excluded from the study.

Sample Collection

Urine samples were collected aseptically from patients diagnosed with urinary tract infections, at, Out Patient Department (OPD) of Department of Urology of KLES Dr Prabhakar Kore Hospital & Medical Research Centre, Belagavi. Samples were transported to the microbiology laboratory of Dr. P. Kore Basic Science Research Centre at 4 -8 °C and processed within 2 hours of collection.

Isolation and Identification of Bacterial Isolates

Urine samples were cultured on Blood agar and MacConkey agar plates using the calibrated loop method. Plates were incubated at 37°C for 24 hours. Colonies exhibiting significant growth were selected and subjected to Gram staining and standard biochemical tests, including catalase, indole, oxidase, Lactose fermentation for bacterial identification.[7]

Screening for ESBL Production

Initial screening for ESBL production was performed using (Table 1, 2), ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic disks (Himedia, Pvt Ltd). Isolates showing reduced susceptibility were confirmed by the combined disk method, where ceftazidime and cefotaxime disks with and without clavulanic acid were used. Increase in the zone diameter of 5 mm or less with clavulanic acid compared to the antibiotic alone indicated ESBL production.[8]

Double Disc Diffusion Test (DDDT) for ESBL Detection

The double disc diffusion method tests for the presence of extended-spectrum β-lactamases (ESBLs) by placing an antibiotic disc (e.g., ceftazidime or ceftriaxone) and a second disc containing the same antibiotic combined with a β-lactamase inhibitor (e.g., clavulanic acid or clavulanate) near the first disc (Himedia Pvt Ltd).

The bacteria producing ESBLs, the β-lactamase inhibitor will restore activity of the antibiotic, increasing the zone of inhibition around the second disc (the combination disc), indicating synergy between the antibiotic and the inhibitor. The increase in zone size of 5 mm or more is considered a positive result for ESBL production.

Meropenem-EDTA Disk Synergy Test (MBL Detection)

This test is used to detect metallo-β-lactamase (MBL) production (Table 1, 3) by using meropenem discs (10 µg) and adding EDTA (a chelating agent) around the antibiotic disc.

EDTA binds to metal ions (like zinc) required for the activity of MBLs, inhibiting the action of the MBL enzyme. If the bacteria produce MBL, the addition of EDTA results in a widening of the zone of inhibition around the meropenem disc, indicating MBL produced by bacteria is chelated by EDTA. A significant increase (≥7 mm) in the zone size when EDTA is present confirms MBL production. All tests were performed to ensure reproducibility and accuracy.[9]

Ethical clearance

The institutional ethical clearance for the study has been obtained from JNMC institutional ethics committee, (Ref No. MDC/JNMCIEC/367, dated 2-07-2024) at J.N. Medical College, Nehru Nagar, Belagavi.

RESULTS

A total of 52 urine samples were collected, out of these 40 yielded *E.coli* and 7 yielded *Klebsiella* sps. The confirmation of isolates (*E. coli* and *Klebsiella* species) was carried out by biochemical tests as per CLSI guidelines. The *E. coli* cultures tested positive for Indole and Catalase, while the Klebsiella species tested negative for Indole and Oxidase. Both bacteria were lactose fermenters, as indicated by the pink coloration on MacConkey agar.

All isolates were then tested for MBL and ESBL production. Two *E. coli* out of 52 isolates were identified as MBL producers, while four *Klebsiella* sps. Out of 7 isolates were identified as ESBL producers. These results were confirmed through the Meropenem (10 mcg)-EDTA Disk Synergy Test for MBL and the ESBL production was checked through Double Disc Diffusion test using Ceftriaxone (30 mcg) and Clavulanic Acid discs (Table 1).

Detection of MBL Production

The *E.coli* isolate No.1 showed zone of 26mm against meropenem antibiotic (10mcq) alone and zone of 34 mm was recorded against meropenem EDTA disc thus showing increase of 8 mm zone confirming MBL production (Table 3). The second isolate *E.coli* displayed zone of inhibition 17 mm against MRP and where as it showed 23mm against MRP (10mcg) with EDTA depicting 7mm increase in zone of inhibition thus confirming MBL production. (Fig.1, 2).

Detection of ESBL Production

The ESBL production was recorded in *E.coli* against CTR by showing zone of 23mm whereas 31 mm zone was observed against CTR with clavulanic acid. There was 8mm increase in zone between CTR and CTR with clavulanic acid thus, showing ESBL production. Further, the isolates 4, 5 and 6 (*Klebsiella sps*) showed the potential ESBL producing capability by showing marked zone of inhibition against CTX along with clavulanic acid. (Fig. 3-6).

Table 1. Results of bacterial isolates that produce ESBL and MBL.

No.	Bacteria	MBL	ESBL
1.	*E. coli*	-	+
2.	*Klebsiella*	-	-
3.	*E. coli*	-	-
4.	*Enterococcus sp.*	-	-
5.	*Staphylococcus aureus*	-	-
6.	*E. coli*	-	-
7.	*Klebsiella*	-	-
8.	*E. coli*	-	-
9.	*E. coli*	-	-
10.	*E. coli*	-	-
11.	*Klebsiella*	-	-
12.	*E. coli*	-	-
13.	*E. coli*	+	-
14.	*E. coli*	-	-
15.	*E. coli*	-	-
16.	*E. coli*	-	-
17.	*E. coli*	-	-
18.	*E. coli*	-	-
19.	*E. coli*	-	-
20.	*E. coli*	-	-
21.	*E. coli*	-	-
22.	*E. coli*	-	-
23.	*Klebsiella*	-	-
24.	*E. coli*	-	-
25.	*E. coli*	-	-
26.	*E. coli*	-	-
27.	*E. coli*	-	-
28.	*E. coli*	-	-
29.	*E. coli*	-	-
30.	*E. coli*	-	-
31.	*E. coli*	-	-
32.	*Klebsiella*	-	-
33.	*E. coli*	-	-
34.	*E. coli*	-	-
35.	*E. coli*	-	-

36.	*E. coli*	-	-
37.	*Klebsiella*	-	-
38.	*E. coli*	-	-
39.	*E. coli*	-	-
40.	*Actinobacteria sp.*	-	-
41.	*E. coli*	-	-
42.	*E. coli*	-	-
43.	*Klebsiella*	-	+

Table 2. ESBL producing bacterial isolates

Isolate	**Bacteria**	**Zone of inhibition** (mm)					
		Antibiotic alone	**Antibiotic+ inhibitor**	**Difference**	**Antibiotic alone**	**Antibiotic+ inhibitor**	**Difference**
		CTR 30mcg	**CTR+ CA**	**CTR & CTR+CA**	**CTX 30mcg**	**CTX+ Clavulanic acid**	**CTX & CTX+CA**
1	*E. coli*	23mm	31mm	8mm	26mm	32mm	6mm
43	*Klebsiella sp.*	10mm	30mm	20mm	13mm	31mm	18mmm

Table 3. MBL producing bacterial isolate

Isolate	**Bacteria**	**Zone of inhibition (mm)**		
		Antibiotic alone (MRP) 10mcq	Antibiotic+ inhibitor (MRP+EDTA)	Difference MRP & MRP+EDTA
13	*E. coli*	26mm	34mm	8mm

MBL and ESBL producing bacteria

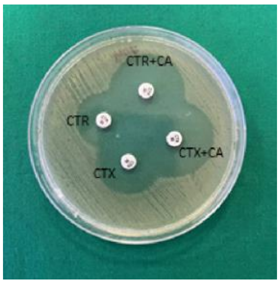

Fig. 1: (001) ESBL positive.

Fig. 2: (013) ESBL positive.

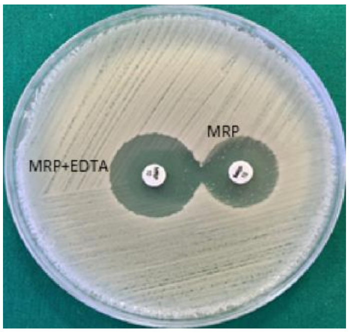

Fig. 3: (043) MBL positive.

DISCUSSION

In the present study low prevalence of ESBL and MBL producing gram negative non-fermentar bacterial isolates, E coli and Klebsiella sp. Only two coliform isolates tested positive for MBL, indicating a low prevalence (3.85%). Four isolates (*E. coli* and *Klebsiella*) tested positive for ESBL, showing a prevalence of 7.69%. No co-existence of MBL and ESBL was observed in any isolate. Our findings are similar to those found in other studies from India [10, 11, 12]. The high proportion of ESBL-negative isolates suggests resistance monitoring is crucial to prevent future resistance spread.

Antimicrobial therapy is threatened by the global rise in resistance, especially in infections with Gram-negative bacteria, where resistance to β-lactams is largely mediated by β-lactamases. Metallo-β-lactamases (MBLs) are encoded both on chromosomes and plasmids, and are widely found in certain non-fermenting bacteria such as *Stenotrophomonas maltophilia*, *Aeromonas species*, and *Chryseobacterium species*.

The present study was aimed at screening and isolating Mettallo β Lactamase and extended spectrum β Lactamase producing bacteria from Urinary tract infected patient's samples.

The bacteria *E. coli* and *Klebsiella* sps. are among the commonest bacteria isolated from clinical specimens. Further, *E. coli* and *Klebsiella sp* are common cause of urinary tract infection[13]. Misuse of antibiotic is responsible for higher incidence of antibiotic resistance among bacteria [14]. Common risk factors associated with infection by multidrug resistant bacteria are hospitalization, inadequate adherence to antibiotic treatment, previous use of antibiotics and self medication [15].

The prevalence of drug-resistant bacteria can differ not only between countries but also among institutions. This variation may be partly due to differences in local antibiotic prescribing practices and the effectiveness of infection control programs across various healthcare settings.

In conclusion, the study highlights low prevalence of ESBL and MBL producing strains, providing opportunity to apply rigorous infection control measures to avoid further increase in resistant isolates. The incidence of Metallo-β-lactamase (MBL) and Extended Spectrum β-lactamase (ESBL) producing bacteria was found to be relatively low in the samples tested with The findings underscore the importance of responsible antibiotic use and infection control measures in reducing the spread of resistance, as misuse of antibiotics, particularly in hospitalized patients, remains a key factor contributing to the development of multidrug resistance.

ACKNOWLEDGMENT

Author's acknowledgment Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education and Research for providing all necessary facilities.

CONFLICT OF INTEREST

The authors state that they have no conflict of interest.

CONCLUSION

The present study it is revealed that *E. coli* isolates produce MBL and *Salmonella Sps*. Produce ESBL and few isolates still showed resistance but they are neither MBL producers nor ESBL producers. The production of MBL and ESBL coupled with multidrug resistance in *E. coli* and *Klebsiella sps.*, is becoming a significant issue in India. The high prevalence of MBL and ESBL production among isolates from outpatients is particularly concerning, indicating the spread of ESBL-producing strains within the rural and urban community. Regular monitoring of MBL and ESBL production, as well as multidrug resistance in these clinical isolates, is crucial. Additionally, implementing a strict policy to regulate antibiotic use is essential to control the rise of drug resistance.

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