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# Prevalence of Resistant Enzymes and Their Therapeutic Challenges

**Vipul Kumar Srivastava[1], Shilpi Sahai[2], Areena Hoda Siddiqui[1]***
[1]Department of Lab Medicine, Sahara Hospital, Lucknow, India
[2]Department of Pulmonary Medicine, Sahara Hospital, Lucknow, India

***Address for Correspondence:** Dr. Areena Hoda Siddiqui, MD, Microbiologist, Department of Lab Medicine, Sahara Hospital, Viraj Khand, Gomti Nagar, Lucknow, India

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**ABSTRACT**- **Purpose:** Multidrug resistant organisms are on the rise. Various enzymes present in the organisms are responsible for this resistance. Detection of these enzymes becomes challenging if organisms harbor multiple enzymes. This study was done to find the prevalence of various enzymes at our tertiary care hospital.

**Materials and methods:** Extended spectrum beta lactamases (ESBL) detection was done by screening method followed by two phenotypic confirmatory methods (double disc synergy and disc potentiation method). Carbapenems (imipenem, meropenem) resistant strain was analyzed for metallo beta lactamases (MBL) and carbapenemases (KPC) using combined disc test and modified Hodge test. Amp C detection was done by using the cefoxitin disc on heavy lawn of *E. coli* ATCC 25922. Distortion of the zone size of the streaked line of the test was taken as positive for Amp C.

**Results:** 87.15% were screened positive for ESBL and confirmed cases were 36.80%. Carbapenem resistant was 31.86%, MBL was 7.52%, KPC was 0.82 %, Amp C in 0.23%.

**Conclusions:** There was high prevalence of ESBL. Detection of these enzymes is important in routine diagnostics for treatment. Co-expression of multiple enzymes was detected in this study. Judicious and rational use of antibiotics is required, which might lead to decrease in the emergence of resistance. Also knowledge of the prevalence of these enzymes helps in empirical antibiotic therapy and in infection control purpose.

**Key-Words-** Multidrug resistant, ESBL, MBL, KPC, Amp C

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## INTRODUCTION

The emergence of multidrug resistance among the pathogens is on the rise and it is posing a serious threat to the management of infections in a hospital care. Initially the most frequently used antimicrobials for empirical therapy were beta lactams. Bacteria produce Beta lactamases, which is responsible for resistance to Beta lactam antibiotics. The first plasmid mediated beta lactamases were TEM-1 (Temoniera-1) and SHV-1 (sulfhydryl "variable") reported in 1965 from *E. coli* and *Klebsiella pneumonia*e [1]. The introduction of third generation cephalosporins in early 1980s particularly ceftazidime and aztreonam after cefotaxime has accelerated the evolution of ESBL worldwide, roughly at the same time and the first report of plasmid encoded beta lactamase capable of hydrolyzing the extended spectrum cephalosporin was published in 1983 from Germany [1-3]. These ESBLs are derived from mutation in older beta lactamases like (TEM-1, TEM2 and SHV-1) and are resistant to third generation cephalosporins (3GCs) and monobactams but are sensitive to cephamycins and carbapenems. They are inhibited by beta lactamase inhibitor combinations (BLI). ESBLs are encoded by transferable conjugative plasmids, which are responsible for dissemination of resistance to other bacteria in the hospital and in the community [2].

Amp C beta lactamases were first discovered in 1970. These organisms are resistant to penicillins, cephalosporins, monobactums, BL/BLI, cephamycins. These are usually sensitive to carbapenems, floroquinolones [4].

The first carbapenemases was identified in 1993.Since then a large number of carbapenemases have been identified, most of them belong to Ambler class A, B, D beta lactamases. True carbapenemases hydrolyse most beta lactams, including carbapenems [5].

KPC-producing *Enterobacteriaceae* were first reported in a clinical specimen from a patient in North Carolina in 2001[6].

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## MATERIALS AND METHODS

A retrospective study was done for a period of 30 months (April 2012 to September 2014) to analyze various enzymes, namely ESBL, Amp C, MBL in a tertiary care hospital. Isolates were obtained from various samples submitted to our lab: urine, respiratory sample, blood, sterile body fluid (CSF, pleural fluid), pus, high vaginal swab. For statistical analysis location was categorized into four groups namely OPD; representing people from community, Emergency; representing admissions from other healthcare area, ICU; representing all critical care areas and wards with stabilized and not serious patients. The study was conducted on non duplicate isolates of *E. coli, K. pneumoneae, K. oxytoca*. Bacterial identification was performed by Vitek2C (Biomereux). For ESBL screening test ceftazidime disc and phenotypic confirmatory used two methods-double disc synergy (ceftazidime, cefotaxime, cefpodoxime, ceftiaxone, amoxiclavulinic acid, Oxoid) (Fig.1) and disc potentiation (ceftazidime clavulanic acid, cefotaxime clavulanic acid combination) (Fig 2). All the isolates resistant to ceftazidime were taken as screening test positive and strains were considered as ESBL positive if either phenotypic confirmatory test was positive [7]. Carbapenems (Imipenem and Meropenem) resistant strains were analysed for metallo beta lactamases (MBL) (Fig. 3) and carbapenemases (KPC) (Fig. 4) using combined disc test using EDTA, Modified Hodge test [7-10]. As there are presently no CLSI or approved criteria for Amp C detection it was performed as a heavy inoculums streaked radially from the cefoxitin disc on the agar surface already streaked with *E. coli* ATCC 25922.Distortion of the zone size was taken positively (Fig. 5) [11]. Quality control used is ATCC *K. pneumoneae* 700603.

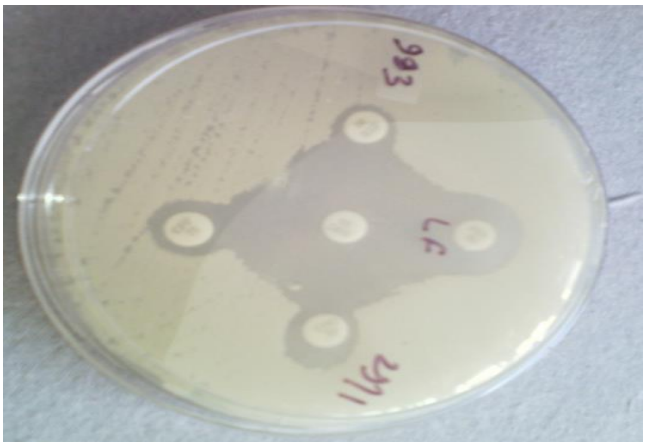

**Fig. 1:** Phenotypic Confirmation Test by Double Disc Synergy Test of Screening Positive Isolate

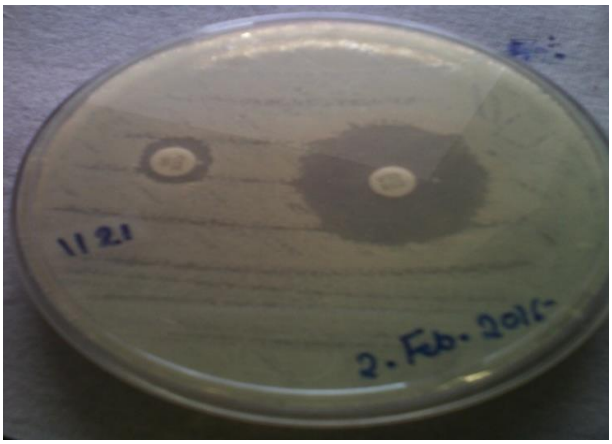

**Fig. 2:** Phenotypic Confirmatory Test by Disc Potentiation Method showing Zone Size of >5 mm in the Disc with Ceftazidime and Clavulanic acid as Compared to Ceftazidime

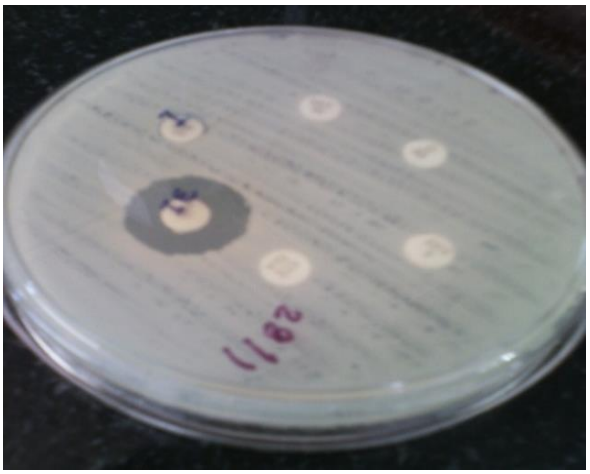

**Fig. 3:** Detection of Mbl (>7 mm Augmentation) by Combined Disc Test

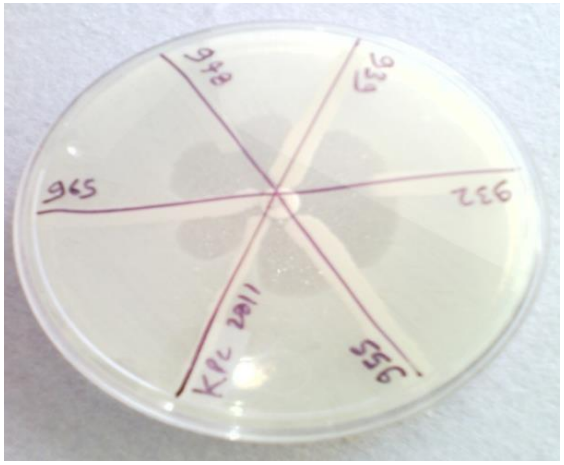

**Fig. 4:** Isolate Showing Carbapenamase (KPC) Enzyme–Modified Hodge Test

 


**Fig. 5: Detection of Amp C. Isolate showing Distortion of Zone Size along the Streaked Line**

## RESULTS

A total of 2584 isolates of *E. coli* and *Klebsiella* species were obtained from April 2012 to September 2014. ESBL positive strains obtained were 951(36.80%), whereas 87.15% (2252/2584) were screening test positive. Amp C was detected in 0.23% (4/1738). Carbapenem resistance were seen in 31.86% (824/2584), MBL 7.52% (91/1210).KPC 0.82% (14/1711).

The prevalence of various enzymes present in different locations are shown in Table 1-4.

**Table 1:** Prevalence of Resistant Enzymes in OPD

| Enzyme | Total Isolates | No. of Isolates Detected | Isolates (%) |
|---|---|---|---|
| **ESBL (Screening test)** | 893 | 666 | 74.58% |
| **ESBL (Confirmed)** | 893 | 365 | 40.87% |
| **ESBL (Not Confirmed)** | 893 | 301 | 33.71% |
| **Amp C** | 658 | 2 | 0.30% |
| **KPC** | 648 | 3 | 0.46% |
| **MBL** | 466 | 15 | 3.22% |
| **Carbapenems Resistant** | 893 | 108 | 12.09% |

**Table 2:** Prevalence of Resistant Enzymes in Emergency

| Enzyme | Total Isolates | No of Isolates Detected | Isolates (%) |
|---|---|---|---|
| **ESBL (Screening test)** | 472 | 439 | 93.01% |
| **ESBL (Confirmed)** | 472 | 247 | 52.33% |
| **ESBL (Not Confirmed)** | 472 | 192 | 40.68% |
| **Amp C** | 294 | 1 | 0.34% |
| **KPC** | 288 | 4 | 1.39% |
| **MBL** | 201 | 8 | 3.98% |
| **Carbapenems Resistant** | 472 | 141 | 29.87% |

**Table 3:** Prevalence of Resistant Enzymes in ICU

| Enzyme | Total Isolates | No of Isolates Detected | Isolates (%) |
|---|---|---|---|
| **ESBL (Screening test )** | 812 | 786 | 96.80% |
| **ESBL (Confirmed)** | 812 | 233 | 28.69% |
| **ESBL (Not Confirmed)** | 812 | 553 | 68.10% |
| **Amp C** | 526 | 1 | 0.19% |
| **KPC** | 517 | 6 | 1.16% |
| **MBL** | 363 | 47 | 12.95% |
| **Carbapenems Resistant** | 812 | 425 | 52.34% |

**Table 4:** Prevalence of Resistant Enzymes in Ward

| Enzyme | Total Isolates | No of Isolates Detected | Isolates (%) |
|---|---|---|---|
| **ESBL (Screening test )** | 407 | 361 | 88.70% |
| **ESBL (Confirmed)** | 407 | 106 | 26.04% |
| **ESBL (Not Confirmed)** | 407 | 255 | 62.65% |
| **Amp C** | 260 | 0 | 0.00% |
| **KPC** | 258 | 1 | 0.39% |
| **MBL** | 181 | 21 | 11.60% |
| **Carbapenems Resistant** | 407 | 150 | 36.86% |

It was found that resistance to ceftazidime was maximum in ICU (96.80%) as shown in Table 3 and least in wards accounting for 26.04% (Table 4). Carbepenem resistance and MBL detection was seen in 52.34% and 12.95% respectively in ICU (Table 3) which was quite high when compared to other areas (Table1&Table 2). Detection of other enzymes Amp C, KPC remained low in all the areas.

All the confirmed ESBL were uniformly sensitive to carbapenems. Not confirmed isolates were found resistant to various agents including aminoglycosides, carbapenems, floroquinolones. Isolates with carbapenem resistance and harbouring other enzymes were sensitive only to polymixins and tigecycline. An isolate was defined as multidrug resistant organism, when found resist to BL, BL/BLI, carbapenems at our institution for infection control purposes.

## DISCUSSION

This study demonstrates the prevalence of resistant enzyme expression in a tertiary care hospital. Enzyme detection is generally not performed in most of the laboratories due to lack of knowledge, lack of facilities to conduct or lack of resources, which can lead to therapeutic failure. ESBL producing Enterobacteriaceae are resistant to cephalospor-

 

ins, aztreonam and monobactam, while resistance to co-trimoxazole and aminoglycosides has been frequently co-transferred on the same plasmid. Many ESBL producing organisms express Amp C beta-lactamases thus conferring resistance to cephalosporins in the 7 alpha-methoxy cephalosporins, oxyimino group, and are poorly inhibited by clavulanic acid. Carbepenems are given for the treatment of infections caused by ESBL producing organisms. With the emergence of carbapenemases and its spread from pseudomonas to Enterobacteraceae, resistance to carbapenems has been noted [12].

Co-expression of multiple ESBL enzymes (CTX-M, TEM, SHV) and at the same time multiple enzymes (AmpC, ESBL, MBL, KPC) are known to occur in a single isolate. These enzymes if present cannot be identified phenotypically, thus making impossible for any lab to identify. In such cases isolates are screening test positive but fail confirmatory tests. These isolates are found resistant to multiple antibiotics including Carbapenems [13-15].

In India, the prevalence rate of ESBL varies in different institutions from 28% to 84% [16] and in our hospital it is 36.80%, which is similar to a study conducted by Bhutada and Shende *et al.* [17]. In a study, done by Grover N *et al.* [4] the prevalence was 40.07% and Amp C was 14.8%. In a study, done by Basavaraj MC the prevalence was 32.1% [18]. In a study by Wattal *et al*. [19] the prevalence of ESBL in *E coli* increased to 61%. In a study, conducted in Mysore the rate was 43% [20].

The prevalence of plasmid mediated Amp C varies widely in different parts of the world from 2% to 46%. In Indian studies, the prevalence of Amp C ranged from 8% to 47% [21]. In our study, Amp C detected very low 0.23%. Various studies have demonstrated the prevalence as 3.3%, 14.8%, and 15.97%, respectively [4,22,23].

The prevalence of carbapenem resistance among isolates reported to the National Healthcare Safety Network (NHSN) in 2006–2007, was up to 4.0% of *E. coli* and 10.8% of *K. pneumoniae* isolates that were associated with certain device-related infections [24]. In a study, done by Datta *et al.* [25], the prevalence of carbapenem resistance was 7.87% and MBL was 5.75%. A study reported high prevalence of resistance to carbapenems ranging from 13 to 51% in *E. coli* and *Klebsiella* sp. from ICUs and wards from a tertiary care hospital in Delhi [19]. Datta *et al.* [25] also reported high prevalence of resistance varying from 17 to 22% to various carbapenems among *Enterobacteriaceae* strains. In a study, carbapenemases detection was15% and 0.5% by combined disc and modified hodge test [26]. In our study, carbapenemases 0.82% and 7.52% by MHT and CDT, respectively. In a study done by Bhutada and Shende *et al.* [17] the pevalance of MBL was 18% and 9.48% [17,20]. The prevalence of KPC in a study by Nayak *et al.* [27] was found to be 16.6%.

In the present study, we found various enzymes prevalent in our set up. Routine screening and confirmatory test should be performed so that appropriate therapy can be chosen for management of patients and containment of infections. Resistant enzymes trend and patterns in different location is important for empirical therapy, epidemiological and infection control purpose. If screening is positive and confirmatory method is negative then possibility of organisms harbouring other enzymes (other than detected) or multiple enzymes should be considered which is more prevalent in critical care areas due to selection pressure. The most active antibacterial agents against Carbapenemases producing with either KPCs or MBLs are colistin, tigecycline [28].

## CONCLUSIONS

Detection, and confirmation of the presence of various enzymes, is important for surveillance, infection control and treatment purpose and to avoid inadvertent use of antibiotics. Challenges are there in the detection of enzymes if multiple enzymes are present in an isolate making it multidrug resistant. Molecular methods are there for identification of various enzymes but they are costly and cost effectiveness should always be kept in mind in the treatment of patients.

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