Original article

Phenotypic detection of ESBL and MBL producing *Klebsiella pneumoniae* in critically ill patients with nosocomial pneumonia

Marwa M. Khalifa¹, Mohamed A El-Mokhtar², Shabaan H Ahmed², Manal A. Mahmoud³, Ehsan M.W ElSabaa*¹

¹- Department of Microbiology and Immunology, Faculty of Pharmacy, Assiut University, Assiut, 71526, Egypt
²- Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, 71515, Egypt
³- Department of Chest Diseases and Tuberculosis, Faculty of Medicine, Assiut University, Assiut, 71515, Egypt

**ARTICLE INFO**

**Article history:**
Received 29 November 2023
Received in revised form 28 December 2023
Accepted 29 December 2023

**Keywords:**
*Klebsiella pneumoniae* nosocomial pneumonia
β-lactamases
MBL
ESBL

**ABSTRACT**

**Background:** Drug-resistant bacteria are major determinants of hospital-acquired infections that are particularly troublesome in intensive care units (ICU). *Klebsiella pneumoniae* (*K. pneumoniae*) is one of the most prevalent causes of nosocomial pneumonia. To overcome the problem of antibiotic-resistant pathogens and stop their spreading, it is crucial to describe the pattern of resistance to antibiotics particularly to the β-lactam group. Therefore, in this study we aimed to determine the rates of extended-spectrum β-lactamases (ESBL) and metallo β-lactamases (MBL) producing *K. pneumoniae* isolates in severely ill hospitalized patients suffering from nosocomial pneumonia.

**Methods:** This was a cross-sectional study that included one hundred patients with hospital acquired pneumonia (HAP) and admitted to Respiratory Intensive Care Unit (RICU) in Assiut University Hospitals, Egypt. Sputum samples were collected and subjected to microbiological analyses to isolate the pathogens. Then, screening, and phenotypic confirmatory tests for ESBL and MBL production among *K. pneumoniae* isolates were carried out.

**Results:** The prevalence of *K. pneumoniae* was 48%. Preliminary screening showed that all isolates were resistant to Ceftazidime (CAZ) and Cefotaxime (CTX), 45/48 (93.75%) were resistant to imipenem (IMP). Confirmatory testing showed that 10/48 (20.83%) isolates were ESBL producers, while 36/48 (80%) isolates were MBL producers and 2/48 (4.17%) were ESBL and MBL producers.

**Conclusion:** The rate of β-lactamases production by *K. pneumoniae* is seriously high, the most frequent type of β-lactamase in *K. pneumoniae* was the MBL, followed by ESBL. The application of infection control measures and antimicrobial stewardship are highly recommended to decrease emergence and spread of drug-resistant strains.

**Introduction**

Hospital-acquired pneumonia (HAP) is an infection of lung parenchyma caused by microorganisms present in the hospital environment. It is a major cause of death and the second most prevalent nosocomial infection [1]. Bacterial infection is the leading cause of HAP among the elderly, specifically *Klebsiella pneumoniae* (*K. pneumoniae*), a Gram-negative bacteria with a capsule that is capable of causing severe pneumonia, particularly in immunocompromised people [2].
The emergence and spread of virulent and multidrug-resistant *K. pneumoniae* represent significant worldwide dangers. Because *K. pneumoniae* has become highly resistant to the most easily accessible antibiotics, it has become a significant superbug. The synthesis of β-lactamase enzymes, that can deactivate β-lactams which is the primary class of drugs used to treat frequent *K. pneumoniae* infections, is part of its MDR arms [3]. As a result, infections caused by *K. pneumoniae* do not respond to standard therapies with increasing morbidity, mortality, and health-care costs [4].

*K. pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* can produce extended spectrum β-lactamases (ESBLs) enzymes. ESBLs are mainly mediated by plasmids and can destroy the β-lactam ring in antibiotic compounds, making them ineffective in stopping pathogen’s growth. Furthermore, these pathogens have the unique ability to hydrolyze aztreonam and oxyiminocephalosporins while being blocked by clavulanic acid, sulbactam, or tazobactam, which are known as “suicide inhibitors” [3].

Due to the rising resistance and significant outbreaks produced by ESBL producing *K. pneumoniae*, carbapenems are now used as a “last resort” antibiotic to cure severe fatal illnesses. However, carbapenem resistance has arisen in *K. pneumoniae*, which is aided by the synthesis of *K. pneumoniae* carbapenemases (KPC), metallo-lactamases (MBLs of GIM, DIM, SIM, NDM, IMP, and VIM subgroups), and carbapenem hydrolyzing oxacillinase (OXA) enzymes [5]. MBLs belong to class B and are capable of hydrolyzing all β-lactams but not monobactams, are resistant to inhibitors of β-lactamase, but are sensitive to chelators of metal ion [6].

Through the years, pathogenic *K. pneumoniae* has developed many resistance mechanisms. The bacterium’s capacity to withstand drugs is a crucial factor in the growth of *K. pneumoniae* infections, particularly in hospital settings where opportunistic antibiotic resistant isolates grow [7].

Although the application of polymerase chain reaction (PCR) for detecting and characterizing ESBL and MBL producers can provide definitive results to medical practitioners but the phenotypic confirmatory tests for ESBL and MBL production are better suited for routine usage in clinical laboratories because of their high sensitivity and specificity. This current study aimed to determine the prevalence of ESBL and MBL production by clinical *K. pneumoniae* isolates from HAP patients admitted to the Chest ICU at Assiut university hospitals, Assiut, Egypt.

**Materials and methods**

**Study participants:**

This was a cross-sectional study that included one hundred critically ill HAP patients admitted to the RICU and only 48 patients fulfilled the inclusion criteria. The study was conducted over a period of 6 months. The research was approved by the Research ethical committee, Faculty of Medicine, Assiut University (IRB: local approval number 04-2023-100025) and was conducted in adherence to the Helsinki Declaration and the World Medical Association's Code of Ethics. Study participants provided informed written consent before the start of this work.

HAP diagnosis was carried out according to the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA, 2016) standards [8]. All enrolled patients were subjected to History taking, physical examination, posteroanterior chest radiographs, complete blood count, kidney function tests, liver function tests, arterial blood gases (ABG) analysis and bacteriologic examination of sputum samples.

**1.1 Inclusion criteria:** Adult patients admitted to RICU for more than 48 hrs.

**1.2 Exclusion criteria:** Samples that showed mixed infection or microorganisms other than *K. pneumoniae* were excluded from the study. Also, patients who were hospitalized during the previous 3 months.

**1.3 Sample criteria:** The good-quality specimens were those with >25 leukocytes and ≤10 squamous epithelium per low power field. Otherwise, it was considered a low-quality specimen [9].

**Isolation and identification of bacterial isolates**

Clinically isolated *K. pneumoniae* were collected from patients with HAP who admitted to the Department of Chest Diseases, Assiut University Hospitals. The samples were cultured on MacConkey agar plates (Himedia, India) and incubated at 37°C for 24 h. *K. pneumoniae* growth was characterized by the presence of pink mucoid colonies [10]. For more characterization the following biochemical assays were performed: citrate utilization test, urease production test and
triple sugar iron agar (A/A with gas) [11]. The American Type Culture Collection (ATCC) reference strain of *K. pneumoniae* ATCC 13883 was taken as a reference strain and was obtained from NAWAH scientific, Egypt.

**Antibiotic susceptibility testing**

The antibiotic susceptibility testing was determined by the Kirby-Bauer method for commonly used antimicrobials. The antibiotic names and their standard inhibition diameters were used as recommended by the clinical and laboratory standards institute 2020 (CLSI, 2020) [12]. Antibacterial discs included amoxicillin (30 µg), amoxicillin-clavulanic acid (20/10 µg), piperacillin (100 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), imipenem (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), amikacin (30 µg), chloramphenicol (30 µg), gentamycin (10 µg).

**Screening test for ESBL producers**

*K. pneumoniae* isolates were screened as ESBL producers by determining their sensitivity to ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic discs (Himedia, India) using disk diffusion method of Kirby-Bauer. Based on the CLSI recommendations, the following were the interpretations of the results: production of ESBL was indicated by inhibition zone of ceftazidime ≤22 mm and cefotaxime ≤27 mm. A confirmatory test was performed on the isolates that were less susceptible or resistant [12].

**Confirmation of ESBL *K. pneumoniae* by combination disk test (CDT)**

Cefotaxime (CTX) or ceftazidime (CAZ) resistant isolates were confirmed as ESBL producers by combination disk test (CDT). Mueller-Hinton agar plates were used for this purpose, along with disks containing 30 µg each of CAZ and CTX, with and without 10 µg clavulanic acid. Discs were placed on each plate with at least 20mm between disc centers. After incubation, the diameter of inhibition zone of every isolate was determined. The isolate was classified an ESBL producer if at least one combination disk was surrounded by an inhibition zone that 5 mm larger than that formed around the opposite antimicrobial disk that didn’t contain clavulanic acid [12].

**Confirmation of ESBL *K. pneumoniae* by double disk synergy test (DDST)**

Mueller-Hinton agar plates with a 30 µg ceftazidime disk and an amoxicillin–clavulanate (containing 10 µg of clavulanate) disk positioned at 30 mm between disc centers. After incubation at 37°C, a resistance to ceftazidime combined with a notable development of ceftazidime inhibition zone in front of the clavulanate-containing disk, the test was considered positive, resulting in a distinctive shape-zone referred to as ‘keyhole’ [13].

**Screening test for carbapenemase producers**

Using the disk diffusion method of Kirby Bauer, the susceptibility of all *K. pneumoniae* isolates to imipenem (10 µg) antibiotic discs (Himedia, India) was used to screen for carbapenemase production. All the isolates of an inhibition zone diameter ≤19mm were considered as positive for carbapenemase production and subjected to MBL confirmation test [12].

**Combination disk test (CDT) for confirmation of MBL *K. pneumoniae***

MBL production was confirmed in imipenem (10 µg) resistant isolates by the IMP-EDTA combination disk test (CDT). This test was performed by placing two disks, one containing imipenem (10 µg) and the other containing imipenem + EDTA (10 µg/750 µg) on Muller-Hinton agar plates that inoculated with *K. pneumoniae*. Discs were placed on each plate with at least 20mm between disc centers and plates were incubated overnight at 37°C. The test was considered positive if the inhibition zone’s diameter around the imipenem+ EDTA disk was 5 mm greater than the inhibition zone's diameter around the imipenem disk [14].

**Results**

**Clinical and laboratory characteristics**

In the present study, 48 *K. pneumoniae* isolates were obtained from sputum samples of 30 (62.5%) males and 18 (37.5%) females. The mean age of our patients was 53.05 years±18.41 (mean±SD). *K. pneumoniae* patients were suffering from different comorbidities for example, pulmonary disease (89.5%), renal impairment (72.9%), diabetes (60.4%), hepatic impairment (58.3%) and ischemic heart disease (8.3%) (table 1).
Frequency of \textit{K. pneumoniae} isolated from sputum samples

In the present study, out of 100 sputum samples, 48 were positive for \textit{K. pneumoniae} growth according to culture characters and biochemical reaction results.

Antibiotic susceptibility testing

As shown in table 2, all isolates were resistant to amoxicillin, amoxicillin-clavulanic acid, piperacillin, ceftazidime and cefotaxime. Various percentages of resistance were reported for ceftriaxone (93%) imipenem (93.8%), aztreonam (87%), ciprofloxacin (93%), levofloxacin (89%), amikacin (91%), chloramphenicol (41.3%) and gentamycin (59%). Out of 48 isolates, 20 (41.7%) were Multi Drug Resistant (MDR) \textit{K. pneumoniae}.

Screening test for ESBL producers

All isolates were ceftazidime (CAZ) and cefotaxime (CTX) resistant then the confirmation of ESBL production was done by combination disk and double-disk synergy methods.

Confirmation of ESBL \textit{K. pneumoniae} by combination disk test (CDT)

By confirmation 10 (20.83%) isolates were ESBL producers. In combination disk test, ESBL producing \textit{K. pneumoniae} isolates showed clear enhanced zone of inhibition around combined CAZ-clavulanic acid and CTX-clavulanic acid discs in comparison to plain disc of ceftazidime and cefotaxime. A breakpoint of \( \geq 5 \) mm was highly effective at distinguishing between ESBLs and non-ESBLs producers (Fig. 1A).

Confirmation of ESBL \textit{K. pneumoniae} by double disk synergy test (DDST)

By confirmation 10 (20.83%) isolates were ESBL producers by the double-disk synergy test. When a decreased sensitivity to ceftazidime combined with a notable enhancement of its inhibition zone in front of the clavulanic acid-containing disk, the test was considered positive, resulting in a distinctive shape-zone referred to as 'keyhole' (Fig. 1B).

Screening test for carbapenemase producers

Out of 48 isolates, 45 (93.75%) were resistant to imipenem (IMP) and then the confirmation of MBL production was done by IPM-EDTA combined disc test.

Combination disk test (CDT) for confirmation of MBL \textit{K. pneumoniae}

By confirmation, 36 (80%) isolates were MBL producers. MBL producing \textit{K. pneumoniae} isolates showed clear enhanced zone of inhibition around combined imipenem and EDTA disc in comparison to plain disc of imipenem (Fig. 2). A breakpoint of \( \geq 5 \) mm was highly effective at distinguishing between MBLs and non-MBLs producers.

Combined Disc test for detection of ESBL & MBL production by \textit{K. pneumoniae} isolates

Out of 48 isolates, 2 (4.17%) were ESBL+MBL \textit{K. pneumoniae}. As shown in (Fig. 3), there were clear enhanced zones of inhibition around both CTX-clavulanic acid disc and imipenem and EDTA disc.

Prevalence of different \( \beta \)-lactamases among \textit{K. pneumoniae} isolates:

Our study showed that MBL-producing \textit{K. pneumoniae} isolates have the highest prevalence of \( \beta \)-lactamases among \textit{K. pneumoniae} followed by ESBL and ESBL+MBL \textit{K. pneumoniae} with prevalence percentage of 93.75%, 20.83% and 4.17%, respectively.
### Table 1. Demographic and laboratory characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>(n=48)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>(62.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>(37.5%)</td>
</tr>
<tr>
<td><strong>Age (years) (mean±SD)</strong></td>
<td>53.05</td>
<td>±18.41</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>29</td>
<td>(60.4%)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>4</td>
<td>(8.3%)</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>43</td>
<td>(89.5%)</td>
</tr>
<tr>
<td>Hepatic impairment</td>
<td>28</td>
<td>(58.3%)</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>35</td>
<td>(72.9%)</td>
</tr>
<tr>
<td><strong>Laboratory data (mean±SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (x 10^9/L)</td>
<td>12.02</td>
<td>±5.7</td>
</tr>
<tr>
<td>Hb (gm/dL)</td>
<td>10.40</td>
<td>±3.55</td>
</tr>
<tr>
<td>PLT (x 10^9/L)</td>
<td>226.23</td>
<td>±31.71</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>11.25</td>
<td>±7.19</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>210.47</td>
<td>±14.32</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>4.9 ±15.12</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>60.83</td>
<td>±36.77</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>39.01</td>
<td>±26.29</td>
</tr>
<tr>
<td>Serum Albumin (gm/dL)</td>
<td>3.44</td>
<td>±5.1</td>
</tr>
<tr>
<td>PaCO2 (mmhg)</td>
<td>43.5</td>
<td>±12.15</td>
</tr>
<tr>
<td>PaO2 (mmhg)</td>
<td>50.03</td>
<td>±9.23</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>78.1</td>
<td>±6.03</td>
</tr>
</tbody>
</table>

WBCs = white blood cell count, Hb = hemoglobin, PLT = platelets, AST = aspartate aminotransferase, ALT = alanine aminotransferase, PaCO2 = partial pressure of carbon dioxide in blood, PaO2 = partial pressure of oxygen in blood, SaO2 = oxygen saturation as measured by blood analysis.

### Table 2. Antibiogram of isolated *Klebsiella pneumoniae*.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>100</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>100</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>100</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>100</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>100</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>93</td>
</tr>
<tr>
<td>Imipenem</td>
<td>93.8</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>87</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>93</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>89</td>
</tr>
<tr>
<td>Amikacin</td>
<td>91</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>41.3</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>59</td>
</tr>
<tr>
<td>Multi Drug Resistant (MDR)</td>
<td>41.7</td>
</tr>
</tbody>
</table>
**Figure 1.** Confirmation of ESBL production (A) combined disc test (A breakpoint of ≥5 mm was highly effective at distinguishing between ESBLs and non-ESBLs producers) (B) double-disk synergy (a notable enhancement of ceftazidime inhibition zone in front of the clavulanate-containing disk, the test was considered positive, resulting in a distinctive shape-zone referred to as 'keyhole'). Cefotaxime (CTX), Cefotaxime-clavulanic acid (CEC), Ceftazidime (CAZ) and Amoxicillin-clavulanic acid (AMC).

![Cefotaxime (CTX) and Ceftazidime (CAZ)](image1)

**Figure 2.** Confirmation of MBL production by combined disc test. MBL producing *K. pneumoniae* isolates showed clear enhanced zone of inhibition around combined imipenem and EDTA disc in comparison to plain disc of imipenem. Imipenem (IMP).

![Imipenem and Imipenem+EDTA](image2)
**Discussion**

According to the World Health Organization, *Klebsiella pneumoniae* is one of the top three priority infections of worldwide concern for public health and a significant nosocomial pathogen. The appearance of multidrug-resistant *K. pneumoniae* isolates, specifically ESBL and, recently, isolates that produce carbapenemase, has put a load on the treatment arsenal. These characteristics are not widely documented in hospital isolates of *K. pneumoniae* in our country. As a result, proper identification of critical virulence genes and antibiotic resistance is required to prevent clinical, therapeutic actions and services from failing [15, 16].

In the present study, the mean age of our patients was 53.05 years where 62.5% of the patients were males and 37.5% were females. The most patients were males probably due to smoking habits which is culturally predominant between males than females. In comparison with our study, Elkholy et al. reported in their study which aimed to analyze the commonest microorganisms responsible for HAP at Tanta University Chest Hospital, Egypt, that the mean age of studied patients was 54.520 years, where 54% of the patients were males and 46% were females [17]. Farhadi et al. reported in their study which aimed to screen frequency of MDR in *K. pneumoniae*, that the mean age of studied patients was 51.7 years, where 58% of the patients were females and 42% were males [18]. Also, Maurici et al. informed that among patients with hospital-acquired respiratory infection, age of patients was 65 years or older, 29.2% were females, while 70.8% were males [19]; there were no significant differences in the average age compared to gender despite males were more than females.

In the present study, *K. pneumoniae* patients were suffering from different comorbidities but the most comorbidity was pulmonary disease (89.5%). This comes in agreement with previous studies which reported that patients those with underlying lung diseases, including chronic obstructive pulmonary disease (COPD), cystic fibrosis are more vulnerable to nosocomial pneumonia owing to abnormalities in the lung structure and function [20, 21].

In the present study, sputum culture had been performed for all studied participants, 48% of the patients had *K. pneumoniae*. This is near to the study done by Mahmoud et al. who reported that the percentage of *K. pneumoniae* causing nosocomial pneumonia isolated from chest intensive care unit (ICU) at Assiut University Hospitals, Egypt, were 50% [22]. In comparison with our study, Negm et al. reported that regarding the sputum culture, the most common pathogens isolated was *K. pneumoniae* with an incidence of 33.51% [23] and Nawara et al. reported that the most frequent organisms causing HAP were *K. pneumonia* with an incidence of 42.9% [24].

Our results revealed that 100% of *K. pneumoniae* isolates were resistant to amoxicillin and piperacillin. The same result was obtained by Farag et al. [25]. In the present study, 100% of *K.
isolates were resistant to amoxicillin-clavulanic acid. In comparison with our study, Mahmoud et al. reported that 90% of K. pneumoniae isolates were resistant to amoxicillin-clavulanic acid [22] and Farag et al. reported that K. pneumoniae resistance to amoxicillin-clavulanic acid was 72% [25].

In the present study, K. pneumoniae resistance to ceftriaxone was 93% while in Negm et al. study, 95.4% resistance to ceftriaxone was observed [23] and Farag et al. showed 89% ceftriaxone resistance [25]. Our study showed that K. pneumoniae isolates were resistant to amikacin (91%) and gentamicin (59%). In comparison with our study, Mahmoud et al. reported 86% amikacin and 54% gentamicin resistances [22] while Negm et al. reported 67.9% amikacin and 72.9% gentamycin resistances [23].

A multidrug resistant (MDR) isolate from the Enterobacteriaceae family, of which K. pneumoniae is a part, is characterized as being resistant to more than one antimicrobial agent in three or more categories of antimicrobial agents, according to Magiorakos et al. [26]. In the present study, 41.7% of K. pneumoniae were MDR isolates. This is in consistence with a study carried out by Maebed et al., who reported that MDR K. pneumoniae isolated from ICU in Beni-Suef University's Hospital, Egypt, represented 40.7% [21]. Higher percentages of MDR K. pneumoniae were reported by Gaballah et al. [27], Farhadi et al. [18] and Mohammed and Anwar [15] (74%, 58% and 59.29%, respectively). The variation in results could be attributed to various isolates, geographical area, sample size, and antibiotics used in infection treatment.

In the present study, 100% of K. pneumoniae isolates were ceftazidime (CAZ) and cefotaxime (CTX) resistant. In comparison with our study, Mahmoud et al. reported that 90% of K. pneumoniae isolated from chest intensive care unit (ICU) at Assiut University Hospitals, Egypt, were resistant to CTX [22], Farag et al. reported that 72% K. pneumoniae isolated from ICU patients in two tertiary hospitals in Cairo, Egypt, were resistant to CAZ and CTX [25], Gaballah et al. reported that 80% and 82% of K. pneumoniae were resistant to CAZ and CTX, respectively, which were isolated from hospital of Medical Research Institute, Alexandria University, Egypt [27].

The double-disk synergy test (DDST) was the first assay created especially to identify Enterobacteriaceae that produces ESBLs. Originally, it was intended to distinguish between isolates that produced ESBLs and bacteria that were resistant to cefotaxime, or that overproduced cephalosporinase [28]. In this study, by confirmation using combination disk and double-disk synergy methods, we found that percentage of ESBL producing K. pneumoniae was 20.83%. This result is in consistence with the study carried out by Shrestha et al. who reported that 23% K. pneumoniae isolates were ESBL producers [29] while higher percentages of ESBL producing K. pneumoniae such as 74.0%, 40%, 78.9% were reported by Mohammed and Anwar [15], Kazemian et al. [30] and Hussein et al. [31], respectively. The other isolates which weren’t confirmed to be ESBL producers may produce other types of β-lactamases such as AmpC β-lactamases and OXA-type β-lactamases [32].

In our study, 93.75% of K. pneumoniae isolates were imipenem (IMP) resistant that is higher than the percentages reported by Mahmoud et al. who reported that 84% of K. pneumoniae isolates were resistant to IMP [22] and Negm et al. who reported that 80.5% of K. pneumoniae isolates were resistant to IMP which were isolated from ICUs in Zagazig University Hospitals, Egypt [23].

In the present study, the confirmation of MBL production using combined disc test was tested in 80% of K. pneumoniae isolates. Imipenem-EDTA Combination disc test was taken as the confirmatory test for MBL. It was regarded as the most sensitive phenotypic test in numerous research and showed MBL percentages near to our percentage such as 91.51% and 88.23% of MBL producing K. pneumoniae reported by Chauhan et al. [33], and Sunita [34], respectively.

The co-occurrence of the two β-lactamases (one isolate produced both ESBL and MBL) was also reported in the present study. In the present study, 2 (4.17%) isolates were ESBL+MBL K. pneumoniae which is near to study done by Kolhapure et al. who reported that 4.81% of K. pneumoniae were ESBL+MBL K. pneumoniae [35]. Higher percentages of ESBL+MBL producing K. pneumoniae such as 11.5% and 32% were reported by Salvia et al. [36] and RaMaKRiShnan et al., respectively[37]. The difference in the prevalence of ESBL and MBL producing K. pneumoniae between the present study and the previous studies could be.
contributed to changes in sample size and clinical specimen type.

Infections caused by *K. pneumoniae* that are resistant to β-lactam drugs due to the development of different enzymes have grown in recent years, making detection of ESBL and MBL critical in both community and hospital isolates. This is because these strains are likely more common than is currently recognized, these enzymes pose a serious danger to currently available antibiotics, and institutional outbreaks are on the rise as a result of selective pressure on the overuse of expanded spectrum cephalosporins and a lack of adequate control measures. As a result, early detection of resistant bacteria infection and adequate antibiotic therapy are required.

Most of the isolates were β-lactam resistant in this study. This is due to an overreliance on β-lactams and other stronger antibiotics for the empirical treatment of illnesses caused by gram negative organisms. The presence of many β-lactamase classes in a single bacterial strain may provide diagnostic and therapeutic problems. The limitation of this study is the inclusion of samples from a single center, and that PCR is not used to detect the genes associated with the resistance due to its high cost.

**Conclusion**

The increased frequency of *K. pneumoniae* in ICUs indicates the significance of early detection of β-lactamase production using basic screening methods that can provide suitable antimicrobial therapy and preventing the development and spread of these multidrug-resistant strains. The current study focuses on the importance of identifying β-lactamases production in *K. pneumoniae* for effective therapy in critically ill patients, allowing us to limit the risk of β-lactamases producers *K. pneumoniae* in hospital settings. Moreover, similar research in specific geographical locations may be encouraged to gain a general understanding of antibiotic susceptibility patterns and β-lactamase formation for optimal management and treatment regimes. Agents with high intrinsic activity against *K. pneumoniae* should be chosen for critically ill patients.

**Conflict of interest**

We declare that we have no conflict of interest.

**Financial disclosures**

Nothing to declare.

**Authorship**

Each author listed in the manuscript had contributed and approved the submission of this version of the manuscript and takes full responsibility for it.

**References**


4- Berglund B, Hoang NTB, Tärnberg M, Le NK, Nilsson M, Khu DTK, et al. Molecular and phenotypic characterization of clinical isolates belonging to a KPC-2-producing strain of ST15 Klebsiella


9- Budayanti NS, Suryawan K, Iswari IS, Sukrama DM. The Quality of Sputum Specimens as a Predictor of Isolated Bacteria From Patients With Lower Respiratory Tract Infections at a Tertiary Referral Hospital, Denpasar, Bali-Indonesia. Front Med (Lausanne) 2019; 6: 64.


17- Elkholy AA, Hantera MS, Abd El-Zaher AH, Mwafy MM, Tourky AS, Abo


34- Sunitha B. DETERMINATION OF METALLO-B-LACTAMASES BY PHENOTYPIC METHODS IN GRAM NEGATIVE BACILLI ISOLATED IN A TERTIARY CARE HOSPITAL. Int J Acad Med Pharm 2023; 5(3): 592-599.

