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Investigation of Antimicrobial Susceptibility Patterns and blaVIM -metallo-β-lactamase Gene in Clinical Samples of Klebsiella pneumoniae

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***Abstract*—Multidrug resistance is a widespread issue that plays an important role in disease outcome. This study was designed to isolate *Klebsiella pneumonia* in different clinical specimens and detected their Antibiotic resistance profile. A total of 319 samples were collected from various clinical specimens for both genders and different ages. The samples were streaked on the blood and MacConkey agars. The bacterial growth identified using biochemical tests and Vitek®2 systems to confirm it. Also, the Vitek®2 system was used to detect the antibiotic sensitivity. Out of 319 clinical samples, K. pneumonia was identified in 67 (21%) cases. The highest isolation rate was in urine 25(37.3%), followed by sputum 13(19.4%), and the least isolation was in CSF with one isolate (1.5%). The results revealed that K. pneumonia isolates were multi-drug resistant pathogens (MDR) with high resistance to Ampicillin (97%) and 85% for piperacillin.**
The PCR results revealed blaVIM- genes frequency was 20 (30%). *K. pneumonia* is one of the bacteria that cause urinary tract infections, and it is a widespread multidrug-resistant pathogen, and blaVIM- producing K. pneumoniae are found in clinical samples at Thi-Qar hospitals. Therefore, monitoring the administration of antibiotics and their rational use is necessary to reduce antimicrobial resistance and treatment failure.

***Keywords*— *Klebsiella pneumonia*, multi-drug resistance, Vitek2 System, PCR.**

I. Introduction

Klebsiella pneumoniae species are capsular bacilli, with Gram- negative staining. They belong to the Enterobacteriaceae family [1].These bacteria were first observed and described in 1882 by a scientist named Karl Friedlander. These bacteria were isolated from the lungs of patients who died of pneumonia. *K. pneumoniae* bacteria are widespread throughout nature, especially in soil, water and animals. They can colonize medical equipment and the environment of healthcare [2].

Moreover, they can be opportunistic pathogens that colonize mucosal surfaces without causing disease However; they can spread from the mucosa to other tissues causing life-threatening infections such as urinary tract infections, pneumonia, bloodstream infections, and sepsis [3]. Depending on its virulent characteristics, *K.pneumoniae* is classified into two categories: classical *K. pneumoniae* (cKp) and hypervirulent *Klebsiella pneumoniae* (hvKp) [4] . Most K. pneumoniae infections belong to the "classical" (cKP) strains, which infect hospitalized patients with chronic diseases requiring long-term care. Classical *Klebsiella pneumoniae* strains have a resistance to various antibiotics, such as carbapenems, [5].

Diversity of virulent factors contributes to *K. pneumoniae* infection. The cardinal significant virulent factors include the mucus capsule, enterotoxins (lipopolysaccharide), siderophores and adhesins. These factors are typically common in the CRKP/hvKp group, causing different immune responses and also the emergence of related phenotypes in hvKp strains [6].

Gram-negative bacteria, *K. pneumoniae*, produce β-lactamase that causes resistance to routine antimicrobials [7]. Metallo-β-lactamases (MBLs) pose a serious threat to recently healthcare. MBLs shows extensive hydrolyze of almost all currently used beta-lactam antibiotics leading to their inactivation. Verona integron-encoded metallo-β-lactamase (VIM) is one of the most common MBLs associated with human infections [8]

Thus, the current study aimed to detect the antimicrobial susceptibility of *K.pneumonia* from clinical isolates, and the frequency of the *bla*VIM -positive gene in *K pneumonia*.

II. Materials and Method

A. Sample collection

Three hundred and nineteen (319) samples of bacteria with *Klebsiella pneumonia* were collected from people in different hospitals, including Al-Hussein Teaching Hospital,

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Al Haboubi Hospital, Al-Musawi Children's Hospital and Bint Al Huda Hospital) and private medical laboratories in the city of Nasiriyah for five months. After growing the samples on the enrichment and differential media, the morphological characteristics of the colonies were observed such as shapes, heights, margins, appearance texture, optical property, and pigmentation of colonies. Gram stain was used to detect all the isolated bacteria, and the isolates were diagnosed using traditional biochemical tests such as the IMVC test and confirmation with the compact system (VITEK-2) for Gram-negative bacteria, prepared by the French company Bio-Merieux.

B. Antimicrobial Susceptibility Test by VITEK-2 Compact System

The VITEK-2 system was used to determine antibiotic sensitivity against several antibiotics by cards of AST. In addition to the detection of bacterial species and genus, CLSI 2023 system was used to interpret the susceptibility of Enterobacteriaceae to different antibiotics [9]

Resistant profile of antibiotic can be either multidrug resistance (MDR) or extensively drug resistance (XDR). In MDR, bacterial isolate is resistant to at least one antibiotic agent in three or more antimicrobial groups.While, in XDR is non-susceptible to one agent at, but two or fewer antimicrobial categories. Pan drug resistance (PDR) is defined as non-sensitive or resistant to all agents in all antimicrobial categories [10] .

C. Extraction of Genomic DNA

Using the manual boiling process described by Yamamoto *et al.*. DNA was extracted from all *Klebsiella pneumoniae* isolates [11].

Inoculation loop was used to pick up one colony from a MacConkey agar and placed in a sterile 1.5 ml Eppendorf tube with 200 µl of distilled water. Then, they were mixed well, and placed on a foam plate and boiled in water for 20 minutes (Analysis of bacteria to release DNA). After boiling, the top and lower parts were mixed upside down, then directly placed in a -20°C refrigerator and frozen for 10 to 12 minutes. This process was repeated three times, and then the Eppendorf tube was centrifuged at 10,000 rpm for 10 minutes. The supernatant was aspirated as template for amplification and stored at −20°C .

D. Detection bla VIM gene by PCR and Electrophoresis

A PCR (Polymerase chain reaction) with specific primer was used to detect the presence of bla VIM gene in bacteria. The primer of bla VIM are F:(5'-TGGTGTTTGGTCGCATATCG 3 R: 5'-AATCTCGTTCCCCTCTACCTC 3´) with amplicon Size (298bp). The amplification run was carried in a final volume of 20µl with 1µl of both Forward Primer and Reverse Primer, 2 µl DNA Template , 6 µl of nuclease free water, Taq Red Master Mix 10 µl (Ampliqon, Denmark). Thermal Cycler (DLAB, China) was run to amplify DNA. The PCR run conditions carried out as the following: ((pre-denaturation at 95 °C for 5 min, 95 °C for 20s, 58 °C for 30s, 35 cycles, and extension at 72° C for 20s)). Ten microliters of the loading dye were mixed with DNA products and analyzed by electrophoresis in agarose gels with 1.5% (Invitrogen, USA) for 45 minute using 1X running buffer (TBE). DNA ladder that was used in each run was 100 bp (SMOBIO Technology, Inc. Taiwan), and DNA bands were observed under UV transilluminator (Akhtarian, Iran) and then photographed [12].

III. RESULTS

In this study, out of 319 clinical samples, Klebsiella pneumonia was demonstrated in 67(21%) cases from different clinical samples. The highest isolates of Klebsiella pneumonia bacteria were around 25 (37.3%) from urine, followed by sputum with 13 (19.4%) isolates, and other sites of isolated bacteria mentioned as shown in Table (1).

Table 1: Prevalence of *K. pneumoniae* on different clinical sources

Type of specimen	*K.pneumoniae* isolates,(%)
Urine Samples	25(37.3%)
Blood Samples	11(16.4%)
Sputum Swab	13(19.4%)
Ear Swab	3(4.5%)
Burn Swab	4(5.9%)
Wound Swab	3(4.5%)
Stool	2(2.9%)
BAL	2(2.9%)
CSF	1(1.5%)
Fluid	3(4.5%)
Total	67(100%)

A. Antimicrobials Susceptibility Test:

The Vitek 2 compact system was used to evaluate the antimicrobial susceptibility to 16 antibiotics included Ampicillin, Amikacin, Azteronam, cefepime, ceftazidime, ceftriaxone, Cefotaxime, Nitrofurantion ciprofloxacin, gentamicin, impenem, meropenem, tetracycline, piperacillin, Piperacillin/Tazobactam and trimethoprim/sulfamethoxazole. The findings of AST (antibiotic susceptibility testing) demonstrated that the highest resistance rate against antibiotics was (97%) for Ampicillin, followed by 85% for piperacillin , 78% for Piperacillin/Tazobactam,75%for trimethoprim/sulfamethoxazole , 71% for azteronam, 70% for ceftazidime, 67% for ceftriaxone, 66% for meropenem and Cefotaxime, 63% for impenem, 61% for cefepime, 57% for ciprofloxacin, 51% for Nitrofurantion,42% for gentamicin, 40% for minocycline, and 35% for Amikacin . In addition to that, intermediate resistance was shown against some antimicrobials such as gentamicin 12%, Nitrofurantion 10%, meropenem 10%, minocycline 8% and trimethoprim/sulfamethoxazole, ceftazidime, ciprofloxacin 3% in some isolates, while other isolates revealed 64% sensitivity against amikacin (Figure 1).

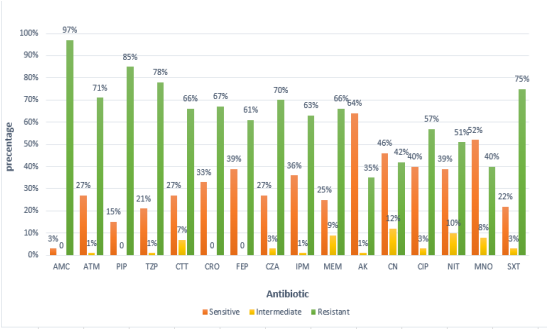

Figure (1): Antibiotic susceptibility of *Klebsiella pneumonia*

B. Drug Resistance Pattern for K. pneumoniae Isolates.

According to the ssusceptibility's results to antibiotics , it revealed that 26 /67(39%) isolates were MDR, 16/ 67 (24%) were XDR , and 12/67 (18%) were finally, 13/67 (19%) were sensitive isolates as shown in Figure (2) .

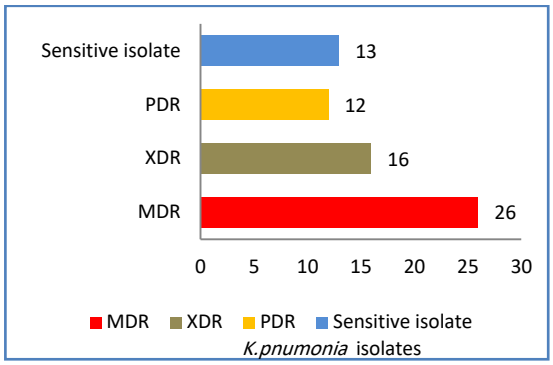

Figure (2): Drug Resistance Pattern for *K. pneumoniae* Isolates

C. DNA Extraction and Identification of bla NDM gene by PCR

The results of DNA extraction indicated that all samples of *Klebsiella pneumoniae* intact genomic DNA as shown in (Figure 3) . Most samples showed good quality and absorption when measured by nano spectrometry(Nanodrob).

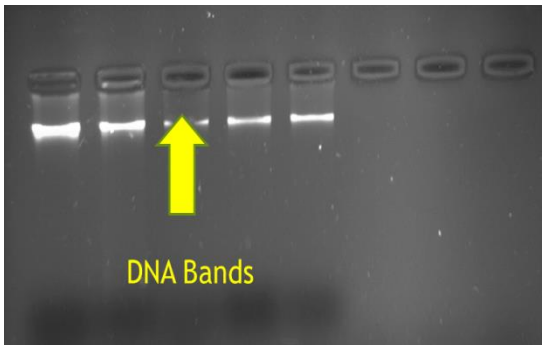

Figure (3): The DNA bands on 0.8% agarose gel at 72 voltages for one hour in 5 samples

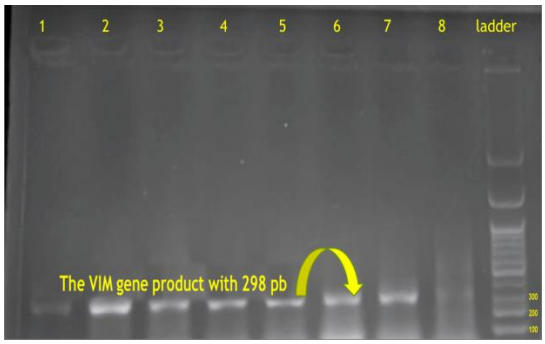

Figure (4): Agarose Gel electrophoresis of *bla* VIM gene(298pb) using specific primers of *K. pneumoniae*. (Ladder: DNA Ladder100- 1500bp. Lanes 1-8, represent bands of *VIM producing K. pneumoniae* isolates). On 1.5%Agarose, 45min at 100v.

The Quality and concentration of DNA specimens were ranged from (1.6-2, and 50-150 ng/ul , respectively). These results demonstrate the efficiency of the extraction method that was used. The current results indicated that VIM was detected in twenty (30%) isolates of Klebsiella pneumoniae . All VIM positive isolates in this study showed PCR product with 298 bp to VIM K. pneumonia specific primer (Figure 4).

IV. DISCUSSION

Most published data indicate that K. pneumoniae is an opportunistic multidrug-resistant (MDR) bacterium that is associated with serious infections such as lung infections, septicemia, urinary tract infections in both hospitals and the community [13].

The current finding showed that the positive infection rate of *Klebsiella pneumonia* was 67(21 %) out of 319 collected clinical samples. The results also indicated that urine samples were the most positive for K. pneumonia bacterial culture with 26(37.3.%) followed by sputum samples with 14(19.4%). The least isolation was in CSF with only one isolate (1.5%) as listed in Table 1. This results agreed with a study conducted in Sulaymaniyah city, northern Iraq which reporting higher percentage of *Klebsiella pneumonia* was in urine sample with (23 42.6%). It is noteworthy that urine infection was common infection in hospitals, while *Klebsiella Pneumoniae* was the second most common bacteria isolated after *E. coli* [14]. Furthermore, previous local study demonstrated that out of fifty positive *Klebsiella pneumoniae* isolates , twenty specimens of urine gave positive culture for *Klebsiella pneumonia* with a great resistance rate to the most antibiotics [15] .

The high presence of these bacteria in urinary tract may be due to the scientific fact that these bacteria located in the flora in the lower digestive tract. In addition to that, their virulence factors contribute to causing their pathogenicity [16].

According to the antimicrobial susceptibility ,the results indicated high resistance rate against ampicillin with (97%) and piperacillin with resistance rate was (85%). This result relatively agreed with local study by Ibrahim *et al.* who noticed that the percentage of resistance to ampicillin was (100%) [17] . These high percentages also agreed with the

results of other study that demonstrated (94.5%) of *Klebsiella pneumonia* isolates were resistant to Ampicillin in Hilla city [18] . The present finding indicated that the rate of resistance to β-lactam antibiotics like Cefotaxime (66%), ceftazidime (70%) and Cefipeme (61%) were less than recent local study by Jwair *et al.* (2023). In their study, they noticed that the high resistant rate to β-lactam antibiotics e.g.: cefazolin (85.0%), ceftazidime (84.0%) and ceftriaxone (83.0%) [19].This relatively high resistance to these antibiotics may be a result of the extensive use of these antibiotics by people without proper medical advice.

The gene that prevalences in this study were blaVIM gene with20/67 (30%) isolates in *K. pneumonia*. These results were higher than a recent study by Jafari-Sales *et al.* who indicated that only 3 (12.0%) isolates of K. pneumonia had the blaVIM gene [20]. The current findings were less than a study done by which found that the gene in 30 (75%) isolates of K. pneumonia [21]. VIM metallo-β-lactamases are factors for failure treatment to bacterial infections, they were first identified in Europe [22] .

In our study, it was found that *Klebsiella pneumoniae* was as an opportunistic multidrug-resistant bacteria(MDR). Some genes for the resistance enzymes bla CTX-M and bla TEM were detected in these bacteria by PCR [23].

V. ETHICAL CONSIDERATION

Ethical permission was obtained from both government hospitals, private laboratories, and all volunteer patients involved in this work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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