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RESEARCH ARTICLE

Frequencies New Delhi Metallo-β-Lactamase (NDM) in Klebsiella pneumoniae Isolates from Clinical Samples in Al-Basrah Governorate, Iraq

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ABSTRACT

Background: Metallo-β-lactamases (MBL) genes are crucial for resistance to antibiotics, and early detection is essential for infection control and prevention of nosocomial outbreaks.

Methods: One hundred fifty clinical samples from Basrah hospitals were collected between October and December 2022 and categorized equally into 50 samples for each sputum, urine, and wound swab. *K. pneumoniae* isolates were identified morphologically and tested on MacConkey and blood agar. The *Klebsiella pneumoniae* chromogenic medium and Vitek®2 system was used as confirmation tests. Genomic DNA extracted from *K. pneumoniae* isolates using a commercial purification kit. The DNA extraction was amplified using PCR for 16S rDNA amplification *K. pneumoniae* isolates using a specific primer of approximately (130bp). *K. pneumoniae* carbapenemase (KPC) chromogenic agar and modified hodge test, according to CLSI were used to test the *K. pneumoniae* isolates for detect the ability of carbapenemase production. Plasmid DNA was extracted from *K. pneumoniae* isolates and plasmid DNA was amplified using PCR to detect the bla_{NDM} gene using a specific primer of approximately (621bp).

Results: From November to December 2022, one hindered fifty samples were investigated for bacterial growth, of which gave 82 (56%) were positive and 68 (45.4%) had negative results. Grame-positive bacteria were 28(34.1%), while Gram-negative bacteria were 54(64.9%), including *Klebsiella pneumonia* 32(59.26%), *E. coli* 16 (29.63%), *Klebsiella spp.* 3 (5.56%), *Pseudomonas spp.* 2 (3.7%), and 1(1.85%) *Proteus spp*. All *K. pneumoniae* isolates showed mucoid pink, white, and purple appearances on MacConkey agar, blood agar, and *K. pneumoniae* chromogenic medium, respectively. The vitek®2 system showed 100% accuracy results in biochemical tests and *K. pneumoniae medium*. The PCR technology was used to diagnose gene 16S rDNA. The results showed that all (n = 32) *K. pneumoniae* isolates had a molecular weight of (130 bp) when compared with the standard molecular DNA ladder (200 bp). On the other side, the (n=32) *K. pneumoniae* isolates tested on *Klebsiella pneumoniae* carbapenemase chromogenic agar and modified Hodge test, 16 (50%) showed positive results and 16 (50%) showed negative results for carbapenemase production in both methods. On the other side PCR molecular diagnostics the bla_{NDM} gene results showed that all (n = 32) *K. pneumoniae* isolates revealed a molecular weight of (621 bp), when compared with the standard molecular DNA ladder (200 bp).

Conclusions: To select the best treatment and avoid losses time and money, use *Klebsiella pneumoniae* carbapenemase (KPC) chromogenic agar, modified Hodge test, and PCR techniques for daily antibiotic susceptibility testing in hospital and private clinical laboratories.

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INTRODUCTION

Klebsiella pneumoniae, an Enterobacteriaceae family opportunistic pathogen, causes nosocomial and community infections.[1] It affects healthy individuals and *often inhabits the* nose, throat, skin, and intestinal tract, but can also cause diseases like pneumonia, urinary tract infections, soft tissue infections, surgical wounds, and sepsis.[2] Due to the existence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP), a multidrug-resistant strain of *K. pneumoniae*, is a serious public health concern on a global level.[3] The misuse and overuse of carbapenems, despite their widespread usage to treat infections brought on by Enterobacteriaceae, has helped speed up the emergence of *K. pneumoniae* isolates that are resistant to these drugs.[4,5] Carbapenem resistance is caused by genes encoding, the most common acquired metallo-β-lactamases (MBLs) include IMP (active on imipenem), VIM (Verona integron-encoded metallo-β-lactamase), SPM (Sao Paulo metallo-β-lactamase), GIM (German imipenemase), SIM (Seoul imipenemase), and NDM (New Delhi metallo-beta-lactamase) enzymes, altering the outer membrane protein (OMP) expression and efflux pumps.

Most resistance is due to carbapenemase production, which hydrolyzes various penicillins, carbapenems, cephalosporins, and aztreonams.[6] MBL genes are found throughout many integrons containing MBL genes that are connected with plasmids or transposons across bacteria. MBL genes play a significant role in the occurrence and are critical for resistance to β-lactam antibiotics in community and hospital settings. Early diagnosis of MBL-producing organisms is critical for infection control and nosocomial outbreak prevention. This study aimed to look for NDM genes in carbapenem-resistant K. pneumoniae isolates from different clinical samples from the Al-Basrah governorate, Iraq.

MATERIALS AND METHODS

Collection of specimens

A total of 150 clinical samples from Basrah hospitals, including Basrah Teaching Hospital Al-Sadder Teaching Hospital, were collected between October and December 2022 and categorized equally into 50 samples for each sputum, urine, and wound swab sample.

Isolation and identification

The *K. pneumoniae* isolates were identified morphologically on MacConkey and blood agar.[7,8] The *K. pneumoniae* chromogenic medium and Vitek®2 system was used as confirmation tests.[9]

DNA extraction

Genomic DNA extracted from *K. pneumoniae* isolates using a commercial purification kit (Presto™ Mining DNA Bacteria, Geneaid, USA) using a manufacturer's protocol.

Detection of 16S rDNA

The DNA extraction was amplified using PCR for 16S rDNA amplification *K. pneumoniae* isolates using a specific primer of approximately (130bp).[10, 11] The resulting PCR product was compared with a standard molecular DNA ladder(2000 bp).

Phenotypic carbapenemase production test

Klebsiella pneumoniae chromogenic medium

The *K. pneumoniae* carbapenemase (KPC) chromogenic agar was used to test the *K. pneumoniae* isolates to detect the ability for carbapenemase production using a manufacturer's protocol.

Modified Hodge Test (MHT)

According to CLSI,[12] the MHT test was performed on all *K. pneumoniae* isolates.

Plasmid DNA extraction

Plasmid DNA that was extracted from *K. pneumoniae* isolates according to (Pure Yield™ Plasmid Miniprep System, Promega, USA).

Amplified the plasmids bla $_{NDM}$

The extracted plasmid DNA was amplified using PCR to detect the *bla$_{NDM}$* gene , using a specific primer approximately (621bp),[13] The resulting PCR product was compared with a standard molecular DNA ladder(2000 bp) .

RESULTS

From November to December 2022, 150 samples were collected and analyzed for bacterial growth. 82 (56%) were positive, while 68 (45.4%) had negative results. Biochemical tests, the Vitek®2 system, and PCR techniques were used. Gram-positive bacteria were 28 (34.1%), while Gram-negative bacteria were 54 (64.9%), including Klebsiella pneumonia 32(59.26%), E. coli 16(29.63%), Klebsiella spp. 3(5.56%), Pseudomonas spp. 2(3.7%), and 1(1.85%) Proteus spp. Figure 1. All (n=32) K. pneumoniae isolate colonies showed mucoid pink, white, and purple-colored appearances on MacConkey agar, blood agar, and Klebsiella pneumoniae chromogenic medium, respectively, the result of vitek®2 system showed the all (n=32) K. pneumoniae isolates achieved 100% accuracy in biochemical test and Klebsiella pneumoniae medium. The results of molecular diagnostics using PCR technology, depending on the diagnostic gene 16S rDNA, showed that all (n=32) K. pneumoniae isolates revealed a molecular weight of 130 bp at a percentage (100%) Figure 2. On the other side, the (n=32) K. pneumoniae isolates tested on K. pneumoniae carbapenemase (KPC) chromogenic agar and the modified Hodge test (MHT) method was used to detect carbapenem-resistant, out of (n=32) K. pneumoniae isolates, 16 (50%) showed positive results and 50 (50%) gave negative results for carbapenemase production in both methods Figures 3 and 4, respectively. On the other hand, the results of molecular diagnostics using PCR technology, depending on the diagnostic the bla$_{NDM}$ gene showed that all (n=32) K. pneumoniae isolates revealed a molecular weight of 621bp at a percentage (100%), when compared with the standard molecular DNA ladder (200bp) Figure 5.

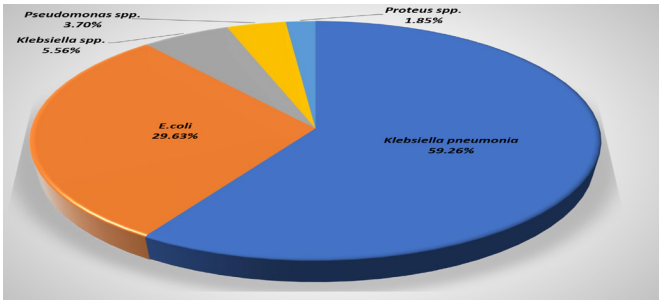

Figure 1: The frequency of gram-negative bacterial Isolates

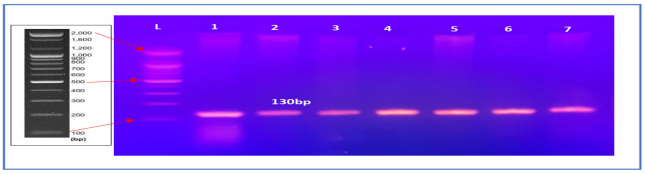

Figure 2: PCR Amplified Products of 16S rDNA. Lane L:(2000 bp DNA ladder), Lane:(no. 1-7) 16S rDNA Band of *K. pneumoniae* Isolates using 1.5% agarose gel, 70V, 45 minutes.

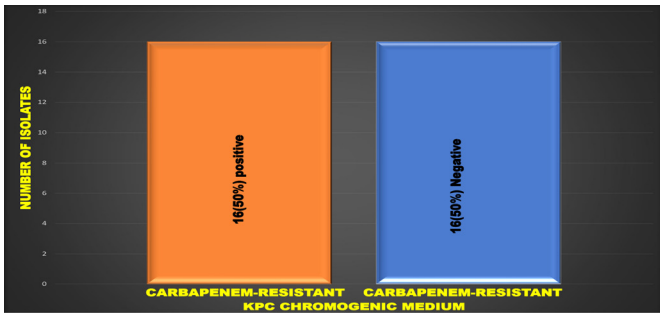

Figure 3: The Results of *K. pneumoniae* isolates Positive and Negative Results for Carbapenemase by Using the *K.pneumoniae* Carbapenemase (KPC) Chromogenic Agar.

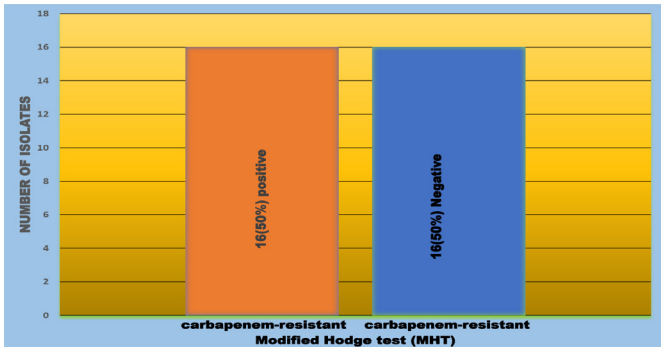

Figure 4: The Results of *K. pneumoniae* Isolates Positive and Negative Results for Carbapenemase by Using the Modified Hodge test (MHT).

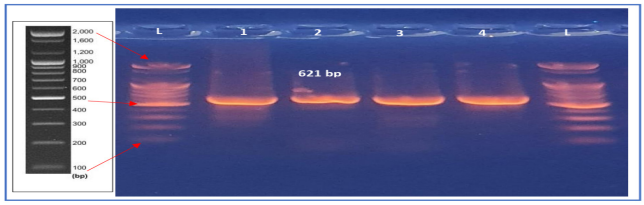

Figure 5: Shows agarose electrophoresis patterns of bla_{NDM} gene PCR amplified products, using 1.5% agarose gel at 70V for 45 minutes.

DISCUSSION

This study reveals a lower prevalence rate of *K. pneumoniae* isolates 32(59.26%) compared to previous studies in Erbil (95.45%),[14] Hilla hospitals (22%),[15] Iran (25%),[16] China (13.3,[17] and Saudi Arabia Hospital (14.7%).18 The increasing isolation rate of *K. pneumoniae* has been observed in China, with a peak in 2020. The study found that *K. pneumoniae* infection was the predominant organism in urine samples 13 (26%), followed by sputum 11 (22%), and wound swabs 8 (16%).[18] This differs from previous studies,[19] which reported 18(36%) *K pneumonia* isolated from sputum, 16(32%) from blood, 9(18%) from urine, and 7(14%)from wound swabs. *K. pneumoniae* is the second most common cause of healthcare-associated UTIs.[20]

On other hand, *K. pneumoniae* isolate have also showed the highest prevalence in urine sample frequency in urine samples, consistent with previous studies in Baghdad (20%).[21] Egypt (50%),[22] and Duhok City (66.2%)[23] .*K. pneumoniae* is a common cause of hospital-acquired pneumonia, with 11(22%)of isolates in the current study from sputum. It is a causative agent in severe infections like surgical-wound infections and septicemia and is associated with ventilator-associated pneumoniae.[24-26] *K. pneumoniae* colonizes mucosal surfaces, leading to hospital-acquired pneumonia.[27] Genotyping is crucial for identifying *K. pneumoniae* cases and tracking infection spread. Genotypic characterization methods are more accurate due to their adaptability to growth conditions, environmental factors, and temperature.[28] 16S rDNA diagnosis is superior to biochemical and phenotypic methods due to its gene presence.[29]

Klebsiella pneumoniae carbapenemase (KPC) chromogenic agar is a reliable method for rapid laboratory detection of carbapenem-resistant.[30] Results show that out of 32 *K. pneumoniae* isolates, 16 (50%) showed positive results and 16 (50%) showed negative results for carbapenemase production. The MHC test is recommended by CLSI[12] for detecting carbapenemase production.[31,32] It is sensitive and specific for *K. pneumoniae* carbapenemases type. Studies of[33] show that (52.17%) of isolates give positive results for MHT, while the study of[32] and[34] show only (17%) and (24%)positive results, respectively. Modified Hodge test detects carbapenemases and New Delhi metallo-β-lactamase (NDM), but its diagnosis depends on PCR. A study[35] in Nigeria found *K. pneumoniae* isolates positive for carbapenemase production on modified Hodge but negative for bla_{KPC} gene on PCR, possibly due to other carbapenemase-producing genes. In current study the results were showed, out of (n=32) K. pneumoniae isolates, the 16 (50%) isolates gave positive results and the 16 (50 %) isolates gave negative results for production of carbapenemase. MHT can be a very useful screening test to suspect such cases for epidemiological purpose.[32]

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