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Research Article

Genotypic detection of CRKP isolates from clinical specimen

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Abstract

The presence of carbapenem resistance isolates was examined technique of PCR. This study showed that blaNDM were the most predominant carbapenemase genes detected in 10 (27%) isolates followed by blaSPM-1 detected in 6 (16.21%) isolates. This study revealed the first report of a blaSPM-1 genes among K. pneumoniae isolates resistance to carbapenem in Najaf. The quick identifications of CRKP and utilizing of adequate disease control measures are essential to inhibit the further infection disseminations by these bacteria in hospitals of Iraq.

Keywords

Klebsiella pneumoniae, carbapenem resistant K. pneumoniae isolates, carbapenem, nosocomial pathogen, Metallo-β-Lactamases.

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Introduction

Klebsiella pneumoniae is one of the main opportunistic and common nosocomial pathogen that causes several extensive and often fatal infections (1). Antibiotics agent may become a difficult task, because K. pneumoniae is intrinsic resistant to several drugs (2). Besides that, K. pneumoniae possesses several mechanisms of antimicrobial resistance through mutations selection in genes of chromosomes or by acquisition of resistant determinants (3). K. pneumoniae easily acquires further resistance pathways to several antibiotics agent, even through the treatment course. Different mechanisms of resistance often exist simultaneously, thus conferring combined resistance (4). Carbapenems have recognized as the most potent beta lactams and widely utilized as the mainstay and empirical for the treatment extensive infections caused by MDR Klebsiella pneumoniae (5). Unfortunately, resistance of K. pneumoniae to carbapenem has now emerged and is spreading worldwide (6). Carbapenem resistance may result from production of carbapenemase enzymes or up-regulation of the efflux pumps (7). Carbapenemases can be divided into major molecular families, when have serine at their active location, referred as serine carbapenemases , which are derivatives of class D or A enzymes and when have at least one atom of zinc as cofactors for optimal activity of enzyme referred as (MBLs), which belong to class B (8). Over the last decade, an ever K. pneumoniae isolates growing number producing MBLs have been showed from several countries ensuring Iraq (9).

Methods and materials

2.1. Separation of whole amount DNA

2.1.1. Technique of boiling

Three to five fresh and pure K. pneumoniae colonies were taken from plate of MacConkey agar into 300 µl of D.W. Then, lysing the colonies by using heat at 100 oC for min in apparatus of Vortex mixer, and then rapidly placing the cells in ice for 30 min, and the other cellular constituents was removed by using centrifuge at 8500 rpm for ten min. Lastly the supernatant was utilized as template of DNA (10). olecular detection of antimicrobial resistance genesproved Standard. On Antimicrobial 25> ,mm for ceftriaxone ,and 27 > mm for

2.2. Applied programs of PCR cycling

Methods of PCR were carried out in a 25 µl volume of reaction, and conditions of PCR amplification performed with a thermal cycler were specific to each set of single primer, based on their on their reference procedure, as follow:

Table (2-2):
Conditions of PCR for class B carbapenemases genes

Gene name	First denaturation	Cycles	Denaturation	Primer annealing	Elongation	Final elongation
bla$_{NDM}$	94ºC / 10 min	36	94ºC / 30 sec	52ºC / 40 sec	72ºC / 50 sec	72ºC / 5min
bla$_{SPM-1}$	95ºC / 2 min	30	95ºC / 30sec	59ºC / 30 sec	72ºC / 70 sec	72ºC / 5min

2.3. Technique of electrophoresis

Gel of agarose was calculated by dissolving 1.5g powder of agarose in 100 ml pf TBE buffer in method of boiling water bath, allowed to cooling of 50˚C, then dye of ethidium bromide (0.5 µl) was added (11).

Result and discussion

3.1: Collections of sample and identifications of K. pneumoniae

In this study, a total number of 650 of different clinical samples were exposed to bacteriological examination for isolating and identifying of Klebsiella pneumoniae isolates. The results of this study reported there were 64 isolates were identified as K. pneumoniae by initial and confirmatory test. Then, data of antibiotic sensitivity for the 64 isolates of K.pneumoniae reported that 37 (57.81%) were resistant to one or both carbapenems.

3.2. Molecular features of carbapenemase genes encoding CRKP isolates

Table (3-2):
Carbapenemase genes profile of carbapenem resistant K. pneumoniae isolates (n=16).

Pattern of combination	NO. (%) of isolates	Isolates code No.
bla_{NDM}	10 (27%)	3, 4, 5, 6, 7, 8,11,14, 18, 19,
Bla_{SPM-1}	6 (16.21%)	5, 10, 11, 12, 13, 14
bla_{VIM}	0 (0%)	0
bla_{KPC}	0 (0%)	0
bla_{GIM}	0 (0%)	0
bla_{SIM}	0 (0%)	0
bla_{IMP}	0 (0%)	0

One of the most significant finding of the current investigation is the presence of blaNDM gene in the collected isolates. According to the results presented here, 10 (27%) carbapenem-resistant NDM-producing K. pneumoniae isolates were identified in table (3-2). This result is the important report of Klebsiella pneumoniae producing blaNDM in Najaf hospitals. Recently, Abbas and Jarallah (2017b) from Babylon reported the first detection of blaNDM in (17.6%) of K. pneumoniae isolates. One general concept from this study may be the endemic dissemination of NDM producing Klebsiella pneumoniae isolates in Najaf, which can be a severe concern. Previous study by Al-Hasnawi (2020) in Najaf showed that 4(18.2%) of Klebsiella pneumoniae isolates produce NDM were identified from a total of 22 CRKP isolates. This study reported that 6 (16.21%) blaSPM-1 producing K. pneumoniae were identified in table (3-2). This result is the initial report of Klebsiella pneumoniae producing blaSPM-1 in hospitals of Najaf. This study is disagreement with recent study done by Al-Hasnawi (2020) in Najaf reported that from a total of 22 carbapenem resistant K. pneumoniae isolates, blaSMP-1 harboring K. pneumoniae were not identified.

Conclusion

This study reported the first detections of blaSPM-1 in CRKP isolates in Najaf. Most of examined CRKP isolates are extensive drug resistance; therefore, such organisms represent a serious therapeutics challenge in patient.

Interest conflict

The authors showed no interest conflict.

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