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Molecular Investigation of Metallo-β-lactamase Encoding Gene in Nosocomial Carbapenem-Resistant Enterobacteriaceae in Iraqi Hospitals

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Abstract: In recent years, there has been an increasing amount of evidence that nosocomial carbapenem-resistant Enterobacteriaceae pose a major puplic health threat. This study aims to uncover the association of multidrug-resistance related genes with increasing the rate of acquired hospital infections by Enterobacteriaceae pathogens. Enterobacteriaceae species (n=57) were detected in clinical specimens (n=45) obtained from patients with infected wounds and burns in 3 Iraqi hospitals. Carbapenem-resistant Enterobacteriaceae bacteria were investigated by antibacterial susceptibility tests. The Molecular investigation by PCR analysis showed that *Klebsiella pneumonia* isolates (n=5) and *Escherichia coli* isolates (n=3) are carrying metallo-β-lactamase encoding gene (bla_{IMP}). It can be concluded that the expression of bla_{IMP} is considered among the main reasons of dominating resistance strains of Enterobacteriaceae pathogens and thus spreading nosocomial infection in Iraqi clinical centers. However, further molecular investigation is needed to overcome this resistance on molecular bases when treating.

Keywords: bla_{IMP}, Enterobacteriaceae, Nosocomial infection, Metallo-β-lactamase

Introduction

Recently, augmented multidrug-resistance among Enterobacteriaceae members has been considered as dreadful medical problem in Iraqi clinical centers (1), (2). Most of the cases with infected burns or wounds including post-operable wounds are administrated with intensive courses of different antibiotics of new generations. However, a number of those patients ended up with bacteremia or eventually septicemia (3).

Enterobacteriaceae represents a group of Gram-negative bacteria that colonise the intestine as either natural microbial flora or pathogens (4). However, varies members belong to this family, such as, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter spp.*, *Proteus spp.*, *Acinetobacter spp*., are among the most common causes of nosocomial infection of burns and wounds (5), (6) Enterobacteriaceae pathogens are implicated in prevalence of antibiotic resistance to a wide range of -lactam therapeutics utilized for treating Enterobacteriaceae related infection. The recent two decades have witnessed emergence of carbapenem-resistant Enterobacteriaceae (CRE) and, therefore, increasing CRE-related infections. This has led to poor prognosis of patients suffering from such infections when treating with -lactams involving therapeutics and therefore increasing rate of morbidities and mortalities (7), (8), (9). There are four main groups of carbapenemases (beta lactamases); Class A carbapenemase (10), Class B carbapenemase (11), Class D -lactamases (12) and carbapenem-hydrolyzing class D -lactamases (13). Metallo-β-lactamase, which is encoded by bla_{IMP} (14). The

reason behind emergence the antimicrobial resistance to carbapenems is that of utilizing carbapenems-related antibiotics to treat the strains producing β-lactamases, which can hydrolyze all -lactams except such antibiotics and then emergence of new generations of strains that can resist this type of antibiotics (carbapenems) (15). The aim of present study is, therefore, to investigate the involvement of bla_{IMP} in prevelance of carbapenem-resistant Enterobacteriaceae in nosocomial infection in Iraqi hospitals.

Method

Specimen Collection

Specimens of infected burns or wounds including post-operable wounds were collected from three local hospitals based in Baghdad, Iraq. There was no need for ethical approval since the samples were autonomously collected from the hospitals' laboratories where the clinical microbiology investigation is routinely carried out. Individuals with infected burns and wound were sampled for four months during the period between January and April, 2018. Sampling of specimens was conducted using swab transporting media.

Bacterial Isolates

Fifty seven isolates of *Enterobacteriaceae* species were diagnosed by the routine automated method Vitek® 2 (BioMérieux, France). The isolates identified comprised 31 *Klebsiella pneumoniae*, 22 *Escherichia coli*, 2 *Enterobacter aerogenes*, 1 *Serratia marcescens* and 1 *Proteus mirabilis*.

Antibacterial Susceptibility

The antibacterial susceptibility tests were carried out for all bacterial isolates using disc diffusion method to identify the carbapenem-resistant Enterobacteriaceae(CRE), while the minimal inhibitory concentrations (MICs) identified by dilution method using Mueller-Hinton broth. For quality control of antibacterial susceptibility test, *E. coli* ATCC 25922 strain was employed.

PCR Detection

Candidate bacterial isolates, which resist or moderately resist imipenem, were subjected to detect *bla*IMP gene by PCR technique using the primers F-5-CTACCGCAGCAGAGTCTTTG-3, R-5-AACCAGTTTTGCCTTACCAT-3 already designed and reported by Senda *et al* 1996 (16).

Results and Discussion

In the present study, three local hospitals based in Baghdad, Iraq were subjected to investigate the prevalence of nosocomial infection with the carbapenem-producing Enterobacteriaceae members. Total of fifty seven bacterial isolates were obtained from forty five clinical samples collected from different sites of body injuries (burn wounds, trauma and post-operable wounds). The specimen collection distributed according the hospital (fifteen from each) and within the same hospital according the type of injury (five from each). 26 of cases were female while the rest 19 were males. Their age ranged between 19-45 years old. 70% of bacterial isolates were obtained from burn specimens followed by trauma accounting for 25%, while the rest (5%) were isolated from post operable wounds.

The bacterial isolates manifested different patterns of response to the tested antibiotics; amikacin, ceftazidime, Cefixime, Cefmetazole, Cefotaxime, imipenem, Norfloxacin and Ampicillin with sulbactam (Table 1). Eight carbapenem-resistant Enterobacteriaceae isolates (five isolates of *Klebsiella pneumonia* and three *Escherichia coli* isolates) were selected for PCR analysis based on imipenem MIC of 4 µg/mL (Table 1). Such isolates are suspicious for production of carbapenemase (Patel *et al* 2017). Therefore, the bla_{IMP} gene was investigated in these isolates.

Table 1. Bacterial susceptibility of blaIMP-expressing isolates

Bacterial isolates	Antibiotic MIC (□g/ml)							
	AN	CAZ	CFM	CMZ	CTX	IPM	NOR	SAM
Klebsiella pneumonia isolates								
KP04	32	>128	>128	128	>128	16	>128	8
KP09	16	64	32	64	>128	16	64	8
KP16	16	>128	128	64	128	64	>128	64
KP18	32	32	>128	>128	64	32	16	4
KP22	16	128	32	>128	64	16	16	128
Escherichia coli isolates								
EC10	>128	>128	>128	>128	>128	128	>128	128
EC22	16	>128	64	128	>128	64	>128	64
EC50	32	128	>128	>128	>128	64	32	64

Abbreviations: AN, amikacin; CAZ, ceftazidime; CFM, Cefixime; CMZ, Cefmetazole; CTX Cefotaxime; IPM, imipenem; NOR, Norfloxacin; SAM, Ampicillin with sulbactam

To screen the resistant isolates, the Clinical Laboratory Standards Institute (CSLI) detention was adopted. CSLI defines Enterobacteriaceae as carbapenem-resistant if they have minimum inhibitory concentrations (MICs) of ≥4 □g/ml against imipenem (17).

The molecular investigation of carbapenem-resistant Enterobacteriaceae by PCR analysis showed that bla_{IMP} gene is carried by four isolates of *Klebsiella pneumonia* (KP04, KP16, KP18 and KP22) in addition to all of the tested *Escherichia coli* isolates (EC10, EC22 and EC50) (figure 1). This may prove the implication of bla_{IMP} in increasing the antibiotic resistance in the examined isolates of hospital acquired infections in Iraq. Nevertheless, the molecular detection of the expression level of metallo-β-lactamase is crucial to solidify this observational evidence.

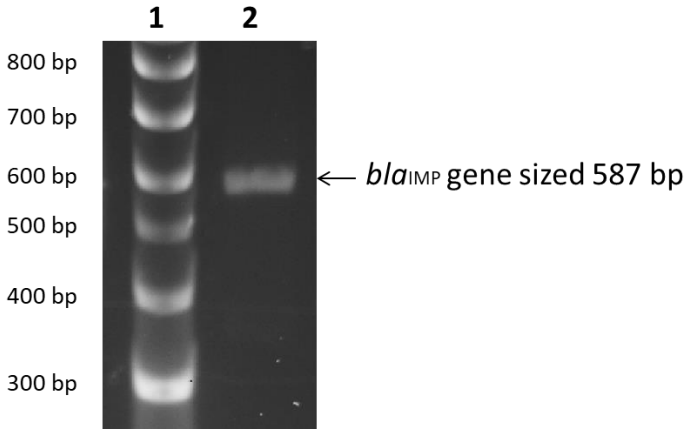

Figure 1: Representative gel electrophoresis photograph showing the amplified sequence of the bla_{IMP} detected in the imipnem resistant isolates. 1) HyperLadder II DNA marker. 2) Amplified DNA fragment of bla_{IMP} gene sized 587 bp.

Conclusion

In a 2013 US Centers for Disease Control and Prevention (CDC) report, carbapenem-resistant Enterobacteriaceae were listed as one of the three most urgent antimicrobial resistant threats. CREs received this highest threat level due to rapidly increasing global spread, propensity for multidrug resistance, and high mortality during blood stream infections (BSI) (18).

Carbapenem resistance in Gram-negative bacteria, especially when carbapenemases are involved, is the main contributing factor for multidrug resistance and usually the definitive step before pan drug resistance. Indeed, resistance to other last-resort drugs among carbapenemase producers may rapidly emerge when these agents are necessarily used in healthcare settings. Moreover, it has been shown that carbapenem-resistant Gram-negative nosocomial pathogens will continue to evolve accumulating more carbapenem-resistance mechanisms, or more than one carbapenemase-encoding gene (19). This will lead in many cases to increased carbapenem MICs ruling

out the best-to-date therapeutic choice against carbapenemase producers, which is the combined treatment including at least one carbapenem. The molecular investigation of such pathogens can be accurate and fast. Implementing of molecular detection of carbapenemase encoding genes in our hospital could bring huge benefits in terms of pridecting the drug of choice to treat the related hospital acquired infection (20). Taking together our and other findings, it can be concluded that the emergence of Enterobacteriaceae nosocomial pathogens pose a foreseeable threat in Iraqi medical centers and, therefore, further molecular analysis is needed to conquer this resistance on expression level when treating.

Recommendations

1. Investigating the molecular mechanisms give rising emergence of Enterobacteriaceae producing carbapenemase may assist to explore how to eliminate spreading these nosocomial pathogens.
2. Employing carbapenemase encoding genes as biomarkers for predicting the antibacterial therapeutic regimens in Iraqi hospitals to reduce the mortalities associated with the antibiotics miss-use to treat Enterobacteriaceae-related nosocomial infections.

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