Original Article

Prevalence of OXA Genes Responsible for Carbapenem-Resistance among *Acinetobacter baumannii* Isolated from Clinical Samples in Iraq

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Abstract

Background: *Acinetobacter*, measured as an opportunistic pathogen has lately occurred as a vital nosocomial pathogen in the world, frequently relating patients with reduced host defenses. Patients in dangerous cases obtain infections while they are staying in care wards, so the incidence of these infections varies significantly in different individuals and clinical settings. **Objectives:** This work aims to examine the prevalence of carbapenem-resistance genes among isolates of *Acinetobacter baumannii* gathered from three hospitals in Iraq. **Materials and Methods:** A total of 30 isolates of *A. baumannii* were gathered from different clinical samples from February to July 2022. DNA of all specimens was extracted. Gradient polymerase chain reaction was applied to discover the genes of bla-OXA carbapenemases. **Results:** The results exhibited a variation of carbapenemase genes in all isolates that had been examined. Although all isolates had at least three genes of carbapenemase that have been tested, the most commonly identified genes in class D β-lactamases were bla OXA-51 and bla OXA-23 which was established in all isolates of *A. baumannii* 30 (100%) tested. It was also found that nine (30%) of tested isolates had borne the gene of bla OXA-58. No isolate exhibited amplification for the gene of bla OXA-40. **Conclusion:** The maximum occurrence and prevalence of the genes of OXA β-lactamase in *A. baumannii* bacteria in Iraqi hospitals were bla OXA-23 and bla OXA-51.

Keywords: *Acinetobacter baumannii*; carbapenem-resistance; Clinical samples; Iraq; OXA genes

Introduction

Diversity of nosocomial infections with in the elevation morbidity and mortality occurred by *Acinetobacter* spp., including bacteremia, pneumonia, urinary tract, skin and soft tissue infections, particularly in severe infection patients.[1] Globally, bacterial infections occurrences are commonly in and out of hospitals,.[2,3] *Acinetobacter* spp. is considered as skin's flora, and a large percentage of healthy individuals are carriers of this pathogen. Also, the hospitalized patients especially when outbreaks happen have higher carriage.[4] Resistance of carbapenem is attributed primarily to molecular class D (OXA enzymes) via OXA-type carbapenemases, and via metallo-β-lactamases to class B enzymes, the resistance is conveyed mostly through chromosomes or through plasmids.[5] OXA51-like β-lactamases inherent to *Acinetobacter baumannii* regarded assay for species specification.[6,7] Prevalence the resistance to carbapenem in *A. baumannii* can be very high in the Middle East.[8] Numerous studies have isolated multidrug-resistant strains and exposed that *A. baumannii* quickly grows resistance to antimicrobials.[9-11]

World Health Organization (WHO) declared that *A. baumannii* is one of multidrug-resistant (MDR) bacteria that have the ability escape excellently from antibacterial drugs.[12] Resistance mechanisms of *A. baumannii* have been recognized extensively via the literature such as the inactivation of the target drugs, modifications of target, pumps of multidrug efflux and defects permeability.[13] Therefore, many community-acquired *Acinetobacter* infections have been recorded and gradually increased worldwide.[14-16]

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Recent studies indicated the presence of antibiotic resistance and virulence factors in *A. baumannii* recovered from animal samples and considered these animals as a possible reservoir of multidrug resistance in these bacteria.[17,18]

The study directed to govern the occurrence of OXA genes accountable for carpenter-resistance in *A. baumannii* isolates improved from clinical specimens.

Materials and Methods

Clinical samples

The intended of current study was to examine the prevalence of carbapenemase genes which bestow carbapenem-resistance in isolates of *A. baumannii* improved from main hospitals in Baghdad, Iraq. Five hundred and forty clinical samples were gathered from patients admitted to three healthcare centers in Baghdad City; Hospital of Ghazi Hariri, Baghdad hospital, Paediatric Teaching Hospital and the National center Laboratories. The samples included urine, wounds, burns, and blood, and were collected during the period from February to July, 2022.

Extraction of DNA and amplification of gene by polymerase chain reaction

On MacConkey agar cultures were grown and subcultured in brain heart infusion broth for extraction of DNA. DNA for isolates of *A. baumannii* was extracted with Kit of Genomic DNA Purification (Promega, Wisconsin, USA). The following primers, bla OXA-58, bla OXA-51, bla OXA-40, and bla OXA-23 were used in this study.[19] In an ultimate volume of 20 µL comprising 5 µL DNA, the polymerase chain reaction (PCR) was performed, 1.5 µL of each forward and reverse primers (Bioneer), 5 µL of Green master mix (Bio Labs) and 7 µL of deionized water, under the following PCR conditions: 94°C/5 min 1×, 94°C/25 s, 52°C/40 s 30×, 72°C/50 s, and with final step 72°C/6 min 30×. By 1.5% agarose gel electrophoresis the

Table 1: Distribution of genes of carbapenemase in *A. baumannii* clinical isolates

Isolate	Genes			
	blaOXA-23	blaOXA-40	blaOXA-51	blaOXA-58
1	+	–	+	+
2	+	–	+	–
3	+	–	+	–
4	+	–	+	+
5	+	–	+	+
6	+	–	+	+
7	+	–	+	+
8	+	–	+	–
9	+	–	+	–
10	+	–	+	–
11	+	–	+	–
12	+	–	+	–
13	+	–	+	+
14	+	–	+	–
15	+	–	+	+
16	+	–	+	–
17	+	–	+	–
18	+	–	+	–
19	+	–	+	–
20	+	–	+	–
21	+	–	+	–
22	+	–	+	–
23	+	–	+	–
24	+	–	+	–
25	+	–	+	+
26	+	–	+	+
27	+	–	+	–
28	+	–	+	–
29	+	–	+	–
30	+	–	+	–
%	100%	%0	100%	30%

products of PCR were separated also stained by 0.5 µg/mL Safe Red (Intron) and visualized using gel documentation.

Ethical approval

Verbal consent was obtained from each patient before taking samples. The study protocol was approved by the committee on publication ethics at the Southern Technical University under the reference No. BMS/0333/021 on October 11, 2021.

Results

Finding the genes of carbapenemase bla OXA-51, bla OXA-23, bla OXA-58, and bla OXA-40 was performed in gradient PCR technique.

From Table 1, results of present study showed a variation of carbapenemase genes in all isolates that had been examined. Although, all isolates had at least three genes of carbapenemase which have been tested, the most

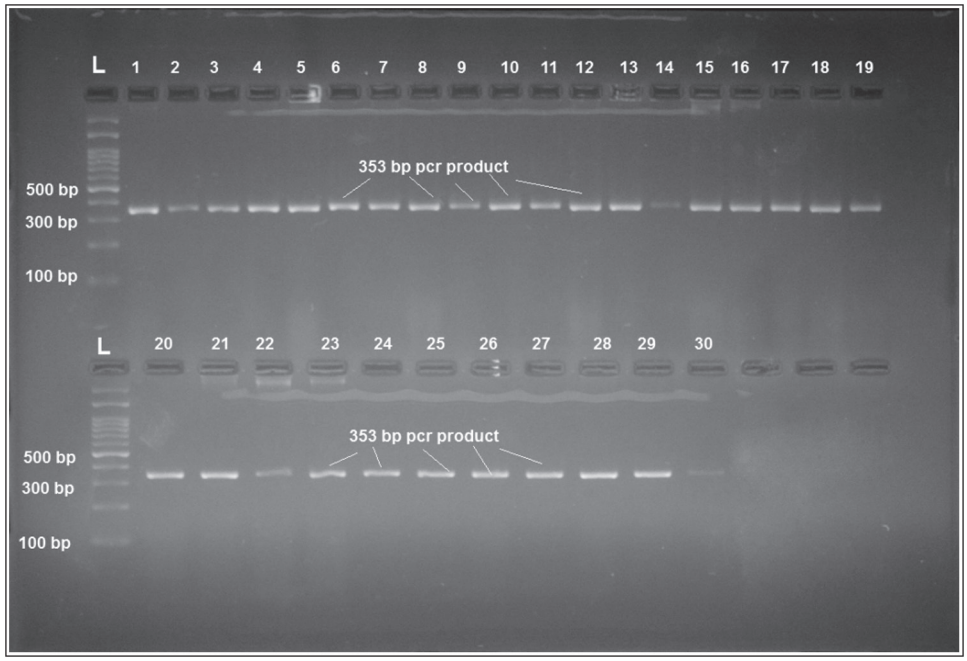

Figure 1: 1.5% Agarose gel with red-safe stained of monoplex PCR-amplified product from extract DNA of *A. baumannii* isolates with *bla*OXA-51 gene product (353 bp). Lane (L), DNA ladder (100 bp). All isolates showed positive results

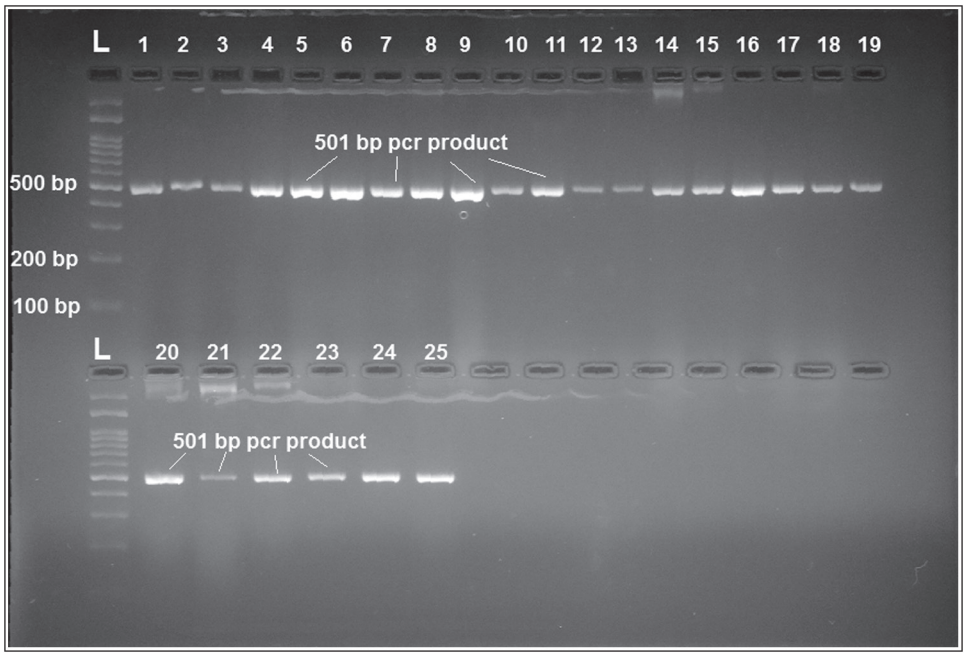

Figure 2: 1.5% Agarose gel with red-safe stained of monoplex PCR-amplified product from extract DNA of *A. baumannii* isolates with *bla*OXA-23 gene product (501 bp). Lane (L), DNA ladder (100 bp). All isolates showed positive results

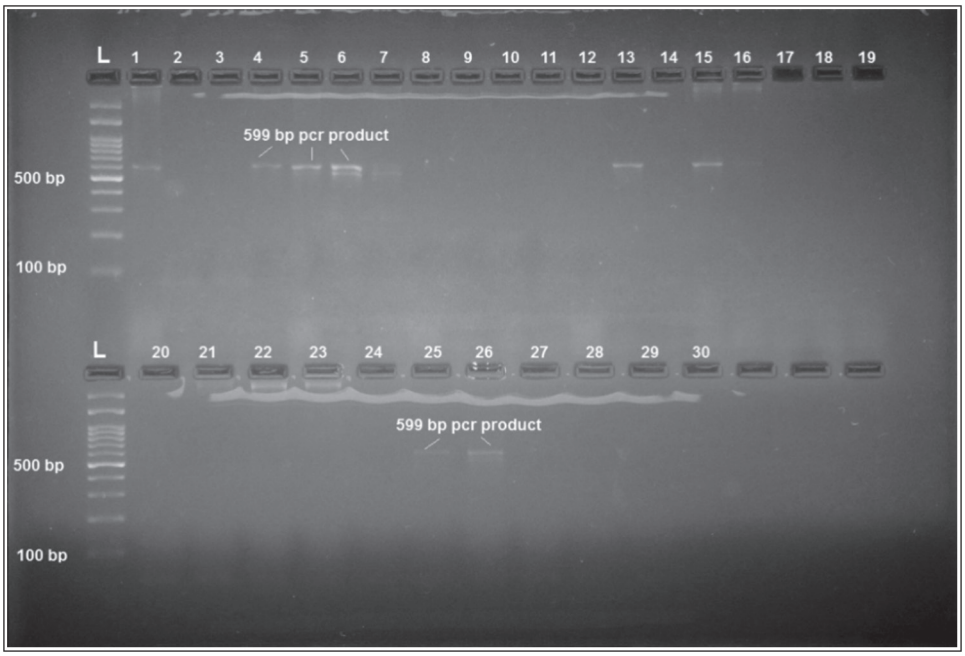

Figure 3: 1.5% Agarose gel with red-safe stained of monoplex PCR-amplified product from extract DNA of *A. baumannii* isolates with *bla*OXA-58 gene product (599 bp). Lane (L), DNA ladder (100 bp). All the isolates showed negative results except 1, 4, 5, 6, 7, 13, 15, 25, and 26 lanes showed positive results

commonly identified genes in class D β-lactamases were bla OXA-51 [Figure 1] and *bla*OXA-23 [Figure 2] which found in all *A. baumannii* isolates 30 (100%) tested. It was also found that 8 (30%) of the tested isolates had been borne *bla*OXA-58 gene [Figure 3], while none of these isolates showed amplification for *bla*OXA-40 gene.

Discussion

All 30 *A. baumannii* isolates in current study (100%) approved chromosomally-encoded *bla*OXA-51-like gene. These results support the findings of other research findings which indicated that the occurrence of the blaOXA-51 gene is a main factor in *A. baumannii* carbapenem resistance,[8] and this result agreed with Egyptian studies declaring the all isolates of *A. baumannii* carried bla OXA-51.[20] In isolates of *A. baumannii* the most prevalent was belong to the oxacillinase from class D serine carbapenemases, OXA enzymes capability to decompose oxacillin is quicker than traditional penicillins.[21] The results revealed that the all isolates were positive for the bla OXA-51 gene (100%). However, several authors reported that *ISaba*1 insertion element may induce an overexpression for OA-23 other than OXA-51.[18]

According to Turton *et al*.,[21] isolates containing *bla*OXA-51, should be resistant to (meropenem and imipenem) if these isolates own *ISAba*1 upstream of *bla*OXA-51. However, our isolates containing *bla*OXA-51, only 14 isolates influenced *ISAba*1 upstream of *bla*OXA-51. Figure 3 showing that all isolates those were positive for *bla*OXA-51 gave bands in 1.5 kb by *ISaba*1F and OXA-51R primers.[22] Other study suggested *bla* OXA-51 accountable carbapenem resistance of such isolates, the evidence of existence for IS*Aba*1 gene by the genes of *bla* OXA so its augmentation the *bla*OXA-51 enzyme approves several studies worldwide,[23] whereas these results were incompatible with other studies which discovered no *ISa*ba1 gene upstream the gene of *bla*OXA.[24,25] Bratu *et al*.,[26] reported that *ISaba*1 is one of several supporters containing IS elements that show a role in the expression of genes that encrypt for antimicrobials resistance. Other enzymes of class D have a bigger affinity for imipenem compared with meropenem.[27] The role of carbapenem resistance seems to be linked with *ISAba*1,[20] the lack of this component, duplicating researches assume a least outcome on vulnerability for carbapenem, unexpectedly in the incidence of an above expressed efflux pump with multidrug properties (Ade ABC).[28] The resistance genes diversity is chiefly upsetting due to the hard choice of experimental therapy of antibiotic in really the patients ill and the potential influence to increase associated costs and hospital stay. In bacterial isolates possessing *bla* OXA-51-like such as the gene of carbapenemase, meropenem and/or imipenem insusceptibility was restricted to the strains having *ISAba*1 upstream to *bla* OXA-51-like.[19] Regarding *bla*OXA-23-like gene, the current result found all strains of *Acinetobacter* (100%) were possessing bla OXA-23-like gene. In contrast with previous local study, it was found that 6 (75%) of CRAB isolates carried *bla*OXA-23,[29] whereas Al-Harmoosh,[30] and Alsehlawi *et al*.,[31] found that 4 (40%) and 2 (40%) of *A. baumannii* strains had positive of bla OXA-23 genes, respectively.

*bla*OXA-23-like gene in isolates of *A.baumannii* also reported from diverse countries, the OXA-23 is considered the first in the resistance among carbapenem OXA-type lactamases recognized in *A. baumannii* bacteria.[32,33] Different countries were exposed to outbreaks caused by the strains of *A. baumannii* which containing bla OXA-23-like genes of carbapenemase.[32,34] From Table 1, it is obvious that nine isolates (30%) demonstrated a positivity to blaOXA-58-like gene. Another one study revealed that this gene is carried by this bacteria and it is noticed from 2% to as high as 84.9 % globally.[35] In contrast, Al-Masoudi *et al*.,[36] in Baghdad, and Vali *et al*.,[37] in Kuwait, did not detect *bla*OXA-58 among their clinical *A. baumannii* isolates. In other studies at Chinese hospitals, no detectable percentage of *bla*OXA-58 genes[38] was recorded. The results from Table 1 showed no one of the *A. baumannii* isolates amplified *bla*OXA-40 genes. The formation member of the OXA-24, was later renamed OXA-40.[39] AL-Harmoosh[30] documented only 30% of *A. baumannii* bacteria harbored *bla* OXA-24 gene.

Conclusion

The maximum occurrence and prevalence the genes of OXA β-lactamase in *A. baumannii* bacteria were bla OXA-23 and bla OXA-51. None of the isolates showed amplification for the gene of bla OXA-40.

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Conflicts of interest

There are no conflicts of interest.

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