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Detection and Characterization of Carbapenem Resistant Acinetobacter *baumannii* Isolated from Different Clinical Specimens in Duhok Province–Iraq

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Abstract

Objective: Acinetobacter baumannii is an opportunistic troublesome pathogen responsible for numerous nosocomial infections, and it commonly develops multi-drug resistance (MDR). The study aimed to assess the carbapenemase production from A. baumannii clinical isolates using various phenotypic and genotypic methods in Dohuk province-Iraq. Methods: A cross-sectional study was conducted on 486 hospitalized and 269 non-hospitalized patients, who were between 10-70 years old, starting from September 2021 through August 2022. The isolated A. baumannii were tested for antimicrobial susceptibility and carbapenemase production via different phenotypic tests. Furthermore, the isolates were screened to detect some carbapenemase- encoding genes through polymerase chain reaction (PCR). Results: A. baumannii was detected in 54 (7.2%) clinical samples, 49(10.1%) were from hospitalized patients, and 5(1.8%) from non-hospitalized patients. The highest percentage was observed in the age group of 51-60 years at 33.3%. The study showed that 94.4% of isolates exhibited multidrug resistance using disc diffusion method, and 66.6% of isolates presented extended drug resistance (XDR). The highest sensitivity rate was seen toward trimethoprim-sulfamethoxazole at 31.5%, followed by levofloxacin and doxycycline at 18.5%. Out of 54 A. baumannii isolates, the modified Carbapenem Inactivation Method (mCIM) reported the highest percentage of sensitivity (98.1%), followed by the Modified Hodge Test (MHT) (94.4%) and Combined Disc Test (CDT) (57.4%). Using PCR, the bla_{OXA-51} genes have been identified in all isolates, while 96.3% of isolates carried bla_{OXA-23} and ISAba1. blaVIM gene was found in 68.5% of isolates only. However, blaNDM, blaIMP, and blaKPC genes were not detected in any isolates. Conclusion: An increase in carbapenem-resistant in A. baumannii is a critical global challenge. The study determined a high carbapenem resistance rate in A. baumannii, commonly by carbapenemase-encoding genes on plasmids. Furthermore, effective infection control procedures and antibiotic programs are required to reduce the spread of these bacteria. The modified carbapenem inactivation method is an excellent test for the early detection of carbapenemase. The study found high prevalence of bla_{OXA-51}, $bla_{OXA-23,}$ and blaVIM resistance genes from isolated A. baumannii, which is a significant alarm.

Keywords
Acinetobacter baumannii, Carbapenem-resistance genes, Oxacilinases, Metallo-beta lactamase, Genotyping, Carbapenemase-encoding genes.

Acinetobacter baumannii (A. baumannii) is a gram-negative, obligately aerobic, coccobacillus

bacterium, which is non-motile, non-lactose fermenter, catalase-positive, oxidase-negative, and has emerged as one of the most common causes of nosocomial infections.[1] A. baumannii has globally appeared as a highly troublesome nosocomial pathogen over the last decades. Its clinical significance has been largely driven by a remarkable capacity to up-regulate or develop a variety of resistance determinants, making it one of the most successful MDR organisms endangering current antimicrobial therapy.[2]

MDR A. baumannii infections were traditionally treated with carbapenems, however excessive use of these drugs has raised the prevalence of carbapenem resistance (CR).[3] As a consequence, in 2017 the World Health Organization (WHO) recognized carbapenem-resistant A. baumannii (CR-AB) as the dangerous, number one priority among a published list of 12 antimicrobial-resistant bacteria that pose a serious threat to modern medicine, highlighted the clinical importance and global burden of infections caused by CR-AB.[4] The primary cause of resistance is overexpression of intrinsic and/or acquired β-lactamases, particularly carbapenem-hydrolyzing class D beta-lactamases (CHDLs).[5,6] In addition, resistance could also be caused by alterations in outer membrane porins, which inhibit the entry of antimicrobials, and overexpression of the efflux pump, which ejects antibiotics, leading to resistance acquisition.[5] There are three molecular subgroups of carbapenemases: Ambler class A (which includes blaKPC and blaGES), Ambler class B (which includes blaIMP, blaSIM, blaNDM, and blaVIM), and Ambler class D (which includes OXA-like or CHDLs) -lactamases, they have spread and appeared everywhere.[7] It's necessary to determine the existence of carbapenemase to evaluate the severity of the problem and to guide the implementation of antibiotic surveillance protocols to stop the spread of non-susceptible carbapenem variants among the A. baumannii strains. Therefore, this study assesses the molecular mechanism of carbapenem resistance in A. baumannii from clinical isolates and examines antibiotic profiles in several hospitals in Duhok province-Iraq.

Materials and Methods

Bacterial isolates

In this cross-sectional study, conducted from September 2021 to August 2022, 755 different clinical samples including (urine, sputum, swab, and blood) were collected, 486 from hospitalized patients and 269 from non-hospitalized patients. Patients ranged in age from 10 to 70 years. The samples were collected in four major hospitals (Azadi Teaching Hospital/Duhok, Golan General hospital/Akre, Dohuk Burn and Cosmetic Hospital, and Emergency Hospital in Duhok) in Duhok province. The samples were cultured on standard laboratory media including MacConkey agar and blood agar, incubated at 37°C for 18-24 hrs. The identification of A. baumannii strains were performed with Gram stain and routine biochemical tests such as indole, catalase, and oxidase. The isolates were bacteriologically confirmed by VITEK 2 (bioMerieux, France). The bacterial isolates were transferred in Brain Heart Infusion broth medium containing 50% glycerol and stored at -80°C. [8]

Antimicrobial susceptibility testing

The antimicrobial sensitivity testing was performed based on Kirby-Bauer disk diffusion method using ciprofloxacin 10µg, levofloxacin 5µg, cefotaxime 30µg, piperacillin 100µg, amikacin 30µg, doxycycline 10µg, tobramycin 10µg, tetracycline 10µg, gentamicin 10µg, meropenem 10µg, imipenem 10µg, trimethoprime-sulphametaxazole 1.25/23.75µg disks (Bioanalyse, Turkey), and results were interpreted according to criteria of clinical and laboratory standards institute (CLSI).[9]

Phenotypic identification of carbapenemase enzyme

Modified Hodge Test (MHT)

The MHT was performed as described previously.[10] A 0.5 McFarland dilution of broth of Escherichia coli ATCC 25922 was prepared. Then diluted to 1:10 and swabbed on Mueller Hinton Agar (MHA) plates. A (10 µg) meropenem disc was placed at the center of the test area. A. baumannii were streaked in a straight line from the edge of the disc to the edge of the plates. The plates were incubated aerobically for 18-24 hrs. at 37 ° C.

Combined Disc Test (CDT)

This test was used for the detection of carbapenemases (Metallo-beta lactamase) expression. The A. baumannii strains were inoculated on MHA plates using a 1:10

dilution of 0.5 McFarland suspensions. Imipenem 10 µg and imipenem/EDTA discs were placed on the plate at least 20mm apart. Zones of inhibition around both discs were compared after overnight incubation at 37°C. A 7 mm increase in the zone of inhibition by the imipenem/EDTA disc over the zone of inhibition around the imipenem disc alone was considered the production of MBLs.[11]

Modified Carbapenem Inactivation Method (mCIM)

The test has been performed according to CLSI guidelines.[9] A loopful of bacterial isolate (A. baumannii) was emulsified in 2 ml of Tryptone Soya Broth (TSB). An imipenem disc (10µg) was soaked in each broth tube and incubated at 37°C for 4 hours. Following the completion of incubation, a 0.5 McFarland suspension of E. coli ATCC 25922 was prepared and inoculated onto MHA plates. Plates were allowed to dry for several minutes. Using a sterile inoculating loop, the disc was removed from the tube and then placed on MHA plates pre-inoculated with the E. coli ATCC 25922 strain. MHA plates were incubated overnight at 37°C in ambient air.

Genotypic determination of carbapenemases production

For fast and easy extraction of bacterial DNA, the conventional boiling method was used. Three to 4 pure colonies were suspended in 500µl deionized water and homogenized by vortex then put in a water bath at 100°C for 15 minutes. After a time, the microtubes were placed in the freezer for 5 minutes. The microbial suspensions were centrifuged at 14,000 rpm for 10 minutes, and the resulting supernatant was added to the new microtubes as a DNA template.[12,13]

The presence of specific gene and carbapenemase genes belonging to class A (blaKPC), class B (blaVIM, blaIMP, and blaNDM), and class D (bla_{OXA-23} and bla_{OXA-51}), and insertion sequence (ISAba1) were investigated in the extracted DNA using PCR. All primers are listed in (Table-1). Following the conditions detailed in (Table-2). PCR products were detected on 1.5% agarose gel (FMC Bioproduct, Rockland, ME) stained with prime safe dye at 80V for 45min-1hr.[14]

Table-1: Primers used in this study

Genes	Nucleotide sequence	Size (bps)
16s rRNA [15]	F: CAGCTCGTGTCGTGAGATGT R: CGTAAGGGCCATGATGACTT	150
bla_{oxa-51} [16]	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	353
bla_{oxa-23} [16]	F: GATCGGATTGGAGAACCAGA R: ATTCTTGACCGCATTTCCAT	501
ISAba1 [17]	F: GTGCTTTGCGCTCATCATGC R: CATGTAAACCAATGCTCACC	430
blaIMP [18,19]	F: GGAATAGAGTGGCTTAAYTCTC R: CCAAACYACTASGTTATCT	188
blaVIM [18,19]	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	390
blaNDM [18,19]	F: CACCTCATGTTTGAATTCGC R: CTCTGTCACATCGAAATCGC	984
blaKPC [20]	F: GTATCGCCGTCTAGTTCTGC R: GGTCGTGTTTCCCTTTAGCC	637

Table-2: PCR amplification conditions of resistance genes.

Genes	Initial denaturation 1 cycle	Denaturation	Annealing 30 cycles	Extension	Final extension 1 cycle
16s rRNA [15]	95°C/3min	95°C/1min	55°C/1min	72°C/1min	72°C/5min
bla_{OXA-51} [16]	95°C/1min	95°C/15s	52°C/15s	72°C/10s	72°C/10min
bla_{OXA-23} [16]	95°C/1min	95°C/15s	52°C/15s	72°C/10s	72°C/10min
ISAba1 [17]	94°C/5min	94°C/40s	54°C/1min	72°C/2min	72°C/5min
bla_{KPC} [20]	94°C/5min	94°C/40s	58°C/1min	72°C/2min	72°C/5min
bla_{VIM} [18,19]	94°C/5min	94°C/40s	54°C/1min	72°C/2min	72°C/5min
bla_{NDM} [18,19]	94°C/5min	94°C/40s	54°C/1min	72°C/2min	72°C/5min
bla_{IMP} [18,19]	94°C/5min	94°C/40s	51°C/1min	72°C/2min	72°C/5min

Ethical approval

This study was approved by the Research Ethical Committee of Duhok Polytechnic University, Kurdistan Region, Iraq, and Duhok General Directorate of Health (Reference No.18082021).

Statistical analysis

Analysis of data was done using the SPSS statistics software (IBM SPSS Statistics Data Editor, version 26). Statistically significant value was considered when p- value≤0.05.

Results

Prevalence of *A. baumannii* among clinical specimens

In the present study, 54(7.2%) A. baumannii

isolates were collected from four hospitals in the Duhok region-Iraq: 49 samples from hospitalized patients and 5 from non-hospitalized patients. A positive relationship was detected between the prevalence of A. baumannii and hospital status of patients (P value <0.0001) (Table-3). Out of 54 A. baumannii isolates, 15 (27.8%) were obtained from urine, swab (wound and burn) n=10 (18.5%), blood n=13 (24.1%), and sputum n =16 (29.6%). A significant relationship was determined between the sample sources and A. baumannii (P value <0.0001). Furthermore, 35(68.8%) of positive cases were among males, while 19(35.2%) were women (P value= 0.142). The prevalence rate of A. baumannii according to age differed from 18/54(33.3%) in the age interval of 51 to 60 years to 1/54(1.8%) in the age interval of 10 to 20 years (Pvalue<0.0001 as in Table 3).

Table-3: Relationship between A. baumannii infection and associated risk factors

	A. baumannii infection (No. (%))				
Demographic data	Positives n=54 (7.2%)	Negatives n=701(92.8%)	Total n=755	χI	Pvalue
Hospital Status					
Hospitalized patients	49(10.1%)	437(89.9%)	486	17.63	<0.0001
Non-hospitalized patients	5(1.9%)	264(98.1%)	269		
Gender					
Male	35(8.4%)	382(91.6%0	417	2.16	0.142
Female	19(4.9%)	319(95.1%)	338		
Age group					
10-20 years	1(0.9%)	114(99.1%)	115	22.73	<0.0001
21-30 years	3(2.4%)	121(97.6%)	124		
31-40 years	8(5.5%)	138(94.5%)	146		
41-50 years	13(10.0%)	117(90.0%)	130		
51-60 years	18(13.3%)	117(86.75)	135		
61-70 years	11(10.5%)	94(89.5%)	105		
Sample source					
Urine	15(3.9%)	366(96.1%0	381	17.77	<0.0001
sputum	16(10.2%)	141(89.8%)	157		
Burns and wound swabs	10(7.4%)	126(92.6%)	136		
Blood	13(16.0%)	68(84.0%)	81		

Antimicrobial susceptibility of *A. baumannii* isolates

Overall, antimicrobial sensitivity testing by disc diffusion reported high resistance rates to most of the tested antibiotics. More specifically, resistance to both imipenem and meropenem were 92.6% and 90.7%, respectively. The resistance rates to Amikacin and piperacillin each 96.3%. Furthermore, 98.1% of isolates exhibit resistance against cefotaxime, 94.4% to tobramycin, 90.7% to gentamycin, and 88.9% to tetracycline. This study reported that several of A. baumannii isolates were susceptible to trimethoprim-Sulfamethoxazole 17/54 (31.5%), followed by levofloxacin and doxycycline each 10/54 (18.5%). This study established that 51(94.4%) isolated bacteria exhibited MDR, and were resistant to at least one antibiotic from three different classes. However, 36 (66.6%) of A. baumannii isolates presented an XDR phenotype, and were resistant to at least one agent in all classes but remained sensitive to only one or two antimicrobial agents as shown in (Table-4).

Table-4: Antimicrobial susceptibility profile of A. baumannii isolates

Antibiotics	Sensitive	Intermediate	Resistant
Piperacillin	2 (3.7%)	0 (0%)	52 (96.3%)
Meropenem	4 (7.4%)	0 (0%)	50 (92.6%)
Imipenem	5 (9.3%)	0 (0%)	49 (90.7%)
Doxycycline	10 (18.5%)	0 (0%)	44 (81.5%)
Levofloxacin	10 (18.5%)	1 (1.9%)	43 (79.6%)
Cefotaxime	1 (1.9%)	0 (0%)	53 (98.1%)
Amikacin	2 (3.7%)	0 (0%)	52 (96.3%)
Gentamycin	5 (9.3%)	0 (0%)	49 (90.7%)
Ciprofloxacin	9 (16.7%)	1 (1.9%)	44 (81.5%)
Tetracycline	6 (11.1%)	0 (0%)	48 (88.9%)
Trimethoprim/ sulfamethoxazole	17 (31.5%)	1 (1.9%)	36 (66.7%)
Tobramycin	3 (5.5%)	0 (0%)	51 (94.4%)
Multidrug resistant	51 (94.4%)		
Extensive drug resistant	36 (66.6%)		

Phenotypic detection of carbapenemases

Fifty-four carbapenem-resistant isolates were screened for carbapenemase production by several phenotypic methods (MHT, mCIM, CDT). The carbapenemase enzyme has been detected in 53(98.1%) and 51 (94.4%) isolates by mCIM and MHT tests, respectively. While only 31(57.4%) of isolates were CDT test positive (Table-5).

Table-5: Phenotypic and genotypic detection of carbapenemase among isolated A.baumannii.

	Positives rate	Negative rate
MHT	51 (94.4%)	3 (5.6%)
CDT	31 (57.4%)	23 (42.5%)
mCIM	53 (98.1%)	1 (1.8%)
Bla_{OXA-51}	54 (100%)	0 (0%)
bla_{OXA-23}	52 (96.3%)	2 (3.7%)
ISAba1	52 (96.3%)	2 (3.7%)
blaVIM	37 (68.5%)1	17 (31.4%)
blaIMP	0 (0%)	54 (100%)
blaKPC	0 (0%)	54 (100%)
blaNDM	0 (0%)	54 (100%)

Molecular detection of genes encoding different carbapenemases and insertion sequences

PCR amplification of A. baumnannii housekeeping gene 16s rRNA gene was identified in 54 isolates (Figure-1). Out of the seven investigated genes, three genes (blaKPC, blaIMP, blaKPC) were not detected in any isolates. Metallo-βlactamase (blaVIM) gene was detected in 37 (68.5%) of isolates. Regarding the genes encoding class D carbapenemases, bla_{OXA-51} gene was detected in all tested isolates and bla_{OXA-23} gene was found in (96.3%). On the other hand, the insertion sequence ISAba1 was identified in all bla_{OXA-23} gene positive isolates (96.3%) shown in (Figure-2) (Tbale-5).

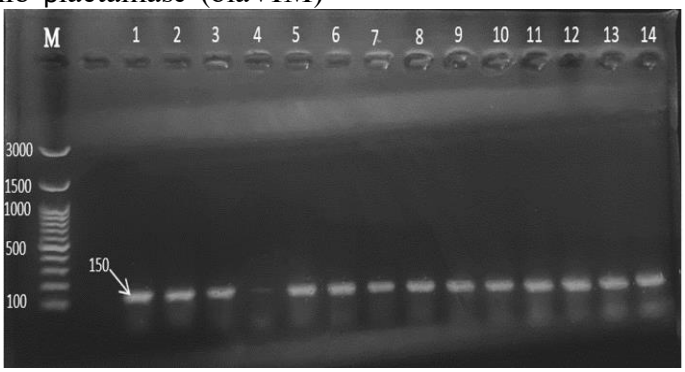

Figure-1: Agarose gel electrophoresis of amplified products of 16s rRNA (150bp) of A. baumannii.

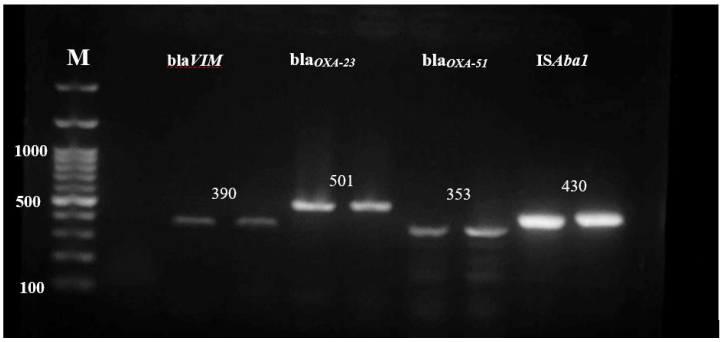

Figure-2: Agarose gel electrophoresis of amplified products of *A. baumannii* carbapenem resistance genes. M: DNA ladder; A: bla*VIM* (390bp); B: bla*OXA-23*(501bp); C: bla*OXA-51*(353bp); D: IS*Aba1*(430bp).

Discussion

Acinetobacter baumannii are gram-negative, saprophytic, coccobacilli bacteria. This pathogen has emerged as a significant nosocomial pathogen due to its ability to survive in hospital environments on a variety of dry and moist surfaces. It is one of the newly emerged bacteria worldwide.[21] This study reported a high prevalence rate of A.

baumannii in hospitalized patients 10.1% compared to non-hospitalized patients 2.8% (P value <0.0001). Similarly, a study by Kim et al [22], reported a higher rate of A. baumannii in hospitalized patients 73.8% than in patients in the community 26.2%. The emergence of A. baumannii infection in hospitals might originate from poor infection-control performance, prolonged hospital stays associated with exposure to antibiotics, contaminated devices, and overcrowding situations, as well as the ability to colonize a large variety of surfaces and biofilm formation. Moreover, mechanical ventilation, surgical interventions, catheter use, increasing age, and co-morbid conditions can increase the risk of infection.[23] A. baumannii is ubiquitous in nature. Many risk factors can increase A. baumannii infections in non-hospitalized patients, such as warm climate which induces the growth of bacteria and enhances virulence-associated gene expression. Therefore, seasonality for community associated A. baumannii colonization is more dominant than healthcare-acquired A. baumannii infection. In addition, excessive alcohol consumption, smoking, or pre-existing conditions such as diabetes mellitus, chronic lung disease, or kidney disease can upsurge the severity of a bacterial infection.[22,24]

According to the gender of the participating patients, the study established that the higher rate of A. baumannii isolates was found in males 68.8%, than in females 35.2%. consistently, studies in Jordan and Iraq have also reported a higher rate in males with 65.6% and 61.2% than in females with 34.4% and 38.8%, respectively.[25,26] The reasons are attributed to the physiological and lifestyle, as well as the immunological state differences between genders.[27]

Regarding the age group of affected patients, the highest rate of isolates was detected in the age interval of 51-60 years at 33.3% followed by 61-70 years at 20.4%, and 41-50 years at 24.1%, while, the lowest percentage was found in age 10-20 years at 1.8%. A positive relationship was detected between the A. baumannii infection and the patient's age group (p value< 0.0001). A recent study conducted in Baghdad-Iraq demonstrated a high rate at 94% of the isolates among patients older than 50 years.[28] In another study in Baghdad, the high proportion of A. baumannii 43.5% was observed in the age group of 40-60 years, which concur with our findings.[29] In comparison to the present study, a study from the United Kingdom revealed that the highest isolation rate among patients older than 35 years of age (70.1%).[30] Older age is one of the risk factors to cause infections of A. baumannii, older patients are more prone to infections for a several reasons including impairment of the structure and functions of certain body systems, as well as, reducing the immune response.[23]

Resistance to broad-spectrum antimicrobial agents in A. baumannii is now an emerging issue globally. In the present study, trimethoprim-sulfamethoxazole was found the most effective antimicrobial against isolated A. baumannii strains, with a 31.5% sensitivity rate, followed by doxycycline and levofloxacin each by 18.5%, while showed a high resistance rate to cefotaxime (98.1%), amikacin and piperacillin each by (96.3%), tobramycin (94.4%), and gentamycin (90.7%), which exhibited the low level of antimicrobials effectiveness against A. baumannii strains. These results are supported by a study conducted in Iraq which showed resistance rate of ciprofloxacin 71 %, levofloxacin 76.3 %, gentamicin 81.5 %, and tobramycin 60.5 %.[31] Additionally, a study in Erbil-Iraq from different clinical sources showed amikacin 78.8%, ciprofloxacin 100%, piperacillin 100%, and gentamicin 96.1%, which were close to this study outcomes to some extent.[32] Furthermore, a study from Turkey reported a lower resistance rate achieved by VITEK2 in amikacin 55.7%, gentamycin 66.9%, and tobramycin 47.5%, while a higher rate in piperacillin 99.6%, ciprofloxacin 94.3%, levofloxacin 95.5%, and trimethoprim-sulfamethoxazole 68.9%.[33] A study in Europe revealed different resistance rates to cefotaxime 93.8%, amikacin 12.5%, ciprofloxacin 18.8%, and gentamicin 50%.[34] This study found that the resistance to imipenem was 90.7% and meropenem was 92.6% by the disk diffusion method. Close to our findings, a previous study in Dohuk-Iraq reported that 80% and 90% of isolates showed

resistance to imipenem and meropenem, respectively.[35] However, another study performed in Erbil-Iraq found a higher resistance rate 100% to the mentioned antibiotics.[36] The variation in studies findings could be due to unreasonable use of antibiotic in a different geographic region which in turn responsible for the development of resistant selective pressure, and differences in susceptibility method (disk diffusion method or VITEK2 system). The high prevalence rate of CR-AB may be due to poor implementation of antibiotic surveillance programs, including the selection of appropriate antibiotics, dose, treatment duration, and control of using antimicrobials; which results in the development of resistance isolates like CR-AB.[37]

Based on the recently accepted standards, MDR and XDR were determined among isolated A. baumannii, MDR is described as resistance to at least one antibiotic in three or more classes of antimicrobial agents, while XDR is described as resistance to at least one antibiotic in all classes but remained susceptible to only one or two antibiotics.[38] This study established that 94.4% of the isolated bacteria displayed MDR and 66.6% of the A. baumannii isolates showed up as XDR. This result is close to a study conducted in Duhok-Iraq by Abdullah and Meraz, which reported 65.9% XDR isolates.[39] Moreover, the majority of A. baumannii isolates 97% were considered to be MDR strains, while 41% were classified as XDR strains in a study from Iran conducted by Salehi et. al., 2018.[40] The increasing A. baumannii resistance rate to routinely utilized antimicrobial agents possibly results from insufficient and inaccurate antibiotics administration, as well as a lack of appropriate infection management procedures.[41] Additionally, the MDR and XDR are attributed to the efflux pumps system and the co-existence of multiple resistance genes on the same mobile genetic elements.[42] In the present study, the ability of MHT test to detect carbapenemase-producing A. baumannii isolates was 94.4%. This finding was in agreement with studies conducted in Egypt and Iraq that recorded a high rate of sensitivity by 94% and 84%, respectively.[10,43] Other studies conducted in Iraq disagreed with this study's results, recording 16.6% and 40.9%.[44,45] The capability of CDT to scrutinize MBLs in isolates was only 57.4% in the current study. Our results concur with previous studies performed in Sulaymaniyah-Iraq and Egypt, in which CDT was able to detect MBLs in 59% and 44.6% of isolates, respectively [46,47]. On the contrary, a study in Egypt reported a higher rate (79.7%) [11]. The carbapenemase enzyme was determined in 98.1% of the tested isolates using the mCIM approach in the present study. The finding was consistent with previous studies in Iraq (100%) and Korea (100%). [45,48]

Oxacillinase-encoding gene (Class D) bla_{OXA-51} was found in all isolated A. baumannii, whereas 96.3% of isolates harbored bla_{OXA-23}. and bla_{OXA-51}. The bla_{OXA-51} is an intrinsic genes and naturally present in A. baumannii chromosomal DNA,[49] while the bla_{OXA-23} gene is transferable and its expression is sufficient to confer CR in A. baumannii.[50] The present study exhibited that the bla_{OXA-23} gene is the most frequent carbapenemase-encoding gene in A. baumannii, in comparison to other screened genes, as detected in previous studies in Baghdad-Iraq (90.74%) and Turkey (94.2%).[51,52] The previous study, by Hou and Yang demonstrated that only 48.38% and 46.31% of isolates harbored bla_{OXA-51} and bla_{OXA-23}, respectively which were in contrast with this study findings.[53]

ISAba1 plays a vital role to provide strong promoter activity when its location is upstream of bla_{OXA-23}, acquiring CR.[54] In the present study, ISAba1 was positive in all bla_{OXA-23}-positive isolates, which was in agreement with prior research from Erbil-Iraq (84.1%).[55] This study finding was inconsistent with study, which showed a rate of 33.3%.[56] As well as, a study in Iran did not detect ISAba1 to be upstream to bla_{OXA-23} (71.1% of positive bla_{OXA-23}), which disagreed with this study findings.[57]

Additionally, this study found that the MBLs-encoding gene (Class B) blaVIM was positive in 68.5% of isolates, which made it the most common MBL. The rapid spread of the blaVIM gene in the A. baumannii strains is probably due to its location on the plasmid, resulting in transformation to the other susceptible bacteria via the plasmid and emerging CR.[58] Studies in

Thi-Qar and Bghdad-Iraq reported similar results in blaVIM with the rate of 65% and 87.9% in isolated A. baumannii. [59,31] Nevertheless, these results disagreed with the study that has been performed in Sulaymaniyah-Iraq, which displayed that only 11.4% of isolates carried blaVIM. [46] In the present study, blaIMP has not been identified in any of the isolated A. baumannii. Similar findings were recorded in Iran [60] and Iraq [43], while blaIMP has been reported in other studies in Iraq with a rate of 30% and 1.9%. [61,62] Furthermore, blaNDM has not been detected in any of the strains in this study; these findings were in contrast with the study in Sulaymaniyah-Iraq and Turkey.[46,63] Contrary, blaNDM was found in 18.8% and 61.5% of isolates in previous studies from India and Iraq.[64,65]

Class A carbapenemases-encoding genes, like blaKPC, have also not been identified in any of the isolated A. baumannii. blaKPC is rarely found in isolates of Acinetobacter spp.[66] These results were in agreement with the prior studies in Kufa and Baghdad-Iraq,[67,68] whereas blaKPC was recorded in a study in Erbil-Iraq, was disagreed with our finding.[69] Here these differences are usually attributed to the differences in environments that facilitate the transmission of mobile genetic elements which in turn attributed to the possible transmission of A. baumannii between different environment sources.[70]

It is important to highlight that the present study identified carbapenemase genes in meropenem and imipenem-sensitive isolates. This might have several explanations: First, this study tested imipenem and meropenem only for screening of carbapenem class against isolated A. baumannii, whilst, these isolates could be resistant to other carbapenem generations, such as ertapenem and doripenem. Second, the presence of the silenced genes not expressed in strains could not be screened by the disc diffusion approach.[46]

The results of compression between phenotypic and genotypic methods exhibited that the most sensitive method was mCIM (98.1%) to detect carbapememase production, followed by MHT (94.5%) and CDT (59.5%). In the current study, the blaVIM gene was not identified in 14.8% of isolates that showed a positive result for CDT, which might be a result of the presence of other genes encoding-MBLs such as blaSIM and blaSPM.[10] Furthermore, the failure of CDT to detect blaVIM in 27.8% of isolates, perhaps due to the low-level activity of the carbapenemase enzyme.[11] Only 51.9% of isolated A. baumannii were positive for all phenotypic approaches.

Conclusion

The study revealed that the prevalence of hospital-acquired infection caused by A. carbapenem-resistant baumannii was much higher than community-acquired, and has become a major cause of healthcare associated infections. The study confirmed the presence of numerous carbapenem-resistant associated genes, and the high prevalence of MDR and XDR A. baumannii in our region, which is a crucial issue to be addressed, proper infection control practices and antibiotics programs are required to minimize the spread of these bacteria in our country. The study established that the mCIM test showed excellent sensitivity and specificity. Further investigations are required from the hospital environment to study the correlation between the efficiency of biofilm formation and exhibiting multiple antibiotic resistances, and also their patterns in clinical and environmental isolates.

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