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Distribution of AmpC-Family genes among *Acinetobacter spp* isolates in Najaf City, Iraq

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Abstract*---Acinetobacter*** species have produced numerous complications in nosocomial infections. AmpC beta-lactamases are regarded as one of the most significant mechanisms of resistance in this pathogen, therefore this study included molecular investigation of AmpC family gene among Acinetobacter spp. in Najaf City, Iraq. The results of culture growth showed that of the 928 affected patients, 487(52.47%) were female and 441(47.52%) were male and 522(56.25%) showed bacterial growth while 406(43.75%) showed no growth. The results revealed that bacterial growth was higher in females 13(4.49%) compared to 15(6.79) in males. Current study recorded 28 (5.36%) Acinetobacter spp. isolates. Recoverd from the different infections, the results of microbial diagnosis showed that 26 isolates were attributed to A. baumannii, while 2 isolates were attributed to A. lwoffii. The PCR data revealed that all *Acinetobacter* spp. isolates in the current study carried 28 (100%) DHA genes, while only 2 (7.14%) isolates from *A. baumannii* carried ACC genes that appeared in positive bands with the correct product size. At the same time, PCR data showed that the genes of CIT, FOX, EBC and MOX were not detected. The sequencing analysis data for the gene DHA were compared with the GenBank database (http://www.ncbi.nlm.nih.gov/Entrez/) and showed that the positive products of PCR amplification returned to the type DHA -1 in all 22 isolates (1 isolate of A. lwoffii and 21 isolates of A. baumannii).

***Keywords*---**PCR, AmpC family, *Acinetobacter baumannii*, *Acinetobacter lwoffii*.

Introduction

Acinetobacter spp. are regarded as significant opportunistic pathogens primarily in immunocompromised people (Van Looveren *et al.*, 2004). *Acinetobacter baumannii* is known to be an opportunistic bacterium that is frequently found in hospitals and produces a variety of infections with fatal effects, it poses a threat to public health due to its high antimicrobial resistance rates and the severe infections it can cause (Zuniga-Moya et al., 2020). *Acinetobacter lwoffii* has been linked to nosocomial infections such as septicemia, pneumonia, meningitis, urinary tract infections, skin, gastroenteritis, and wound infections in hospitals. (Hu *et al.*, 2011; Rakitin *et al.*, 2021). Antimicrobial resistance has emerged as a significant and growing phenomena, resulting in rising healthcare expenses around the world. It has been linked in recent years to high morbidity, death, and increased costs as a result of both prolonged hospitalization and treatment (Kyriakidis *et al.*, 2021).

Transposons IS elements, plasmids, and bacteriophages are frequently related with antibiotic resistance in Acinetobacter spp.. (Rakitin *et al.*, 2021). AmpC-lactamases are cephalosporinases that belong to the class C or group I family and are resistant to alpha methoxy-lactamases like cefoxitin or cefotetan. These -lactamases give resistance to narrow, extended, and broad spectrum cephalosporins, as well as -lactam/-lactamase inhibitor combos, hence their identification is therapeutically significant. (Bush *et al.*, 1995; Kumar et al., 2014). There are currently six types of ampC gene families found on plasmid-mediated plasmids: EBC, MOX, ACC, CIT, FOX, and DHA (Moehario et al., 2019). Currently, no or little information is available regarding hospitalized patients in Najaf-City / Iraq about AmpC-types genes producing Acinetobacter spp. so an Acinetobacter species producing AmpC-*-lactamase was examined in this study.

Materials and Methods

Collection of the specimens and Bacterial Isolation and Identification

A total of 928 clinical specimens were collected from patients admitted to the main hospitals and privates clinical laboratories in Najaf City , these specimens involved wound swabs (109), burns swabs (71), sputum (70) , ear swabs (63), blood (54), seminal fluid (44), pretoeneal fluid (19), pleural fluid (13), throat swab (41), vaginal swab (32) and urine (412) .All specimens were cultured immediately on blood agar and MacConkey agar and incubated aerobically in 37oC for 24 hour (MacFaddin, 2000)**.** Morphological characters and biochemical test were used to diagnosis the bacteria and all suspected *Acinetobacter* spp. were confirmed based on Vitek-2 system

DNA extraction and PCR assay

Total genomic of 28 isolates of Acinetobacter spp. were extracted using genomic DNA extraction mini kit (Favorgen, Biotech, Corp. Korea), the extraction was performed based on the guidance of manufacture corporation. The nucleic acid was conserved under -20^{o}C state using the deep freezing device, the PCR technique was employed to examine and detect all the genes described in table

(1). The gel document (Cleaver, United Kindom), was applied to migration of PCR products at 1% agarose (iNtRoN, Biotech. Inc., Korea), after dye of ethidium bromide at 0.5 µg/ml concentration was applied (Al-Hamdani and Tuwaij, 2020).

Table (1)
Conditions and sequence of primers used in this study

Gene name	Primer Sequence 5' to 3'	Annealing	Size of product	Reference
CIT-F	TGGCCAGAACTGACAGGCAAA	64^0C	462 bp	Kolar *et al.*, (2020)
CIT-R	TTTCTCCTGAACGTGGCTGGC			
MOX-F	GCTGCTCAAGGAGCACAGGAT	64^0C	520 bp	
MOX-R	CACATTGACATAGGTGTGC			
DHA-F	AACTTTCACAGGTGTGCTGGGT	64^0C	405 bp	
DHA-R	CCGTACGCATACTGGCTTTGC			
ACC-F	AACAGCCTCAGCAGCCGGTTA	64^0C	346 bp	
ACC-R	TTCGCCGCAATCATCCCTAGC			
EBC-F	TCGGTAAAGCCGATGTTGCGG	64^0C	302 bp	
EBC-R	CTTCCACTGCGGCTGCCAGTT			
FOX-F	AACATGGGGTATCAGGGAGAT	64^0C	190 bp	
FOX-R	CAAAGCGCGTAACCGGATTGG			

Sequence analysis of DHA gene

Twenty-two isolates of *Acinetobacter* spp. that showed positive band with PCR amplification were chooses and were sent to company of Macrogen (Korea) to detected and analyzed the sequence of nucleotides. The data of sequence were compared with the results in the GenBank database through using BLAST at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/). Forward DNA strands of target gene was sequenced according to procedure of Sanger method (the dideoxy chain termination).

Results

The results of culture growth indicated that among 928 patients involved 487(52.47%) female and 441(47.52%) male were 522(56.25%) bacterial growth compared with 406(43.75%) no growth. The results listed in Table 2 indicated that although the number of bacterial growth in females was above that of males, the percentage of *Acinetobacter* spp. isolates in females were 13(4.49%) compared with 15(6.79) in male from a total of 28(5.36%) *Acinetobacter* spp. isolates.

Table (2)
Distribution of *Acinetobacter* spp. between gram negative according the gender

gender	specimens growth		Gram-negative		*Acenitobacter* spp.	
	number	%	number	%	number	%
female	298	57.08	221	74.16	13	4.49

male	224	42.91	172	76.78	15	6.69
total	522	100	393	75.28	28	5.36

Data of the present study showed a heterogeneous distribution of *Acinetobacter* spp. isolates among the different infections of the patients, whereas the results of microbial diagnosis showed that 26 isolates were returned to *A. baumannii* compared with 2 isolates were returned to *A. lwoffii*. The number of pathogens recorded according to the source of infection detailed in table 3.

Table (3)
Distribution of *Acinetobacter* spp. according the source of infection

Sample source	Total	growth	Acinetobacter		Total
			lwoffii	baumannii	
Wound swab	109	101	1	9	10
Urine	412	184	1	3	4
Sputum	70	41	0	5	5
Ear swab	63	38	0	0	0
Blood	54	19	0	1	1
Burn	71	62	0	8	8
Seminal fluid	44	17	0	0	0
Pretoeneal fluid	19	2	0	0	0
Pleural fluid	13	2	0	0	0
Throat swab	41	32	0	0	0
Vaginal swab	32	24	0	0	0
Total	928	522	2	24	28

PCR data was revealed that all *Acinetobacter* spp. isolates in the current study were harbored 28(100%) DHA gene, while only 2 (7.14%) isolates of A. baumannii were harbored ACC gene which appeared in positive bands at the correct product size figure 1 and 2). At same respect, PCR date revealed that genes of CIT, FOX, EBC and MOX were no detected in this study. Data of sequencing analysis for DHA gene were compared with database of the GenBank (http://www.ncbi.nlm.nih.gov/Entrez/) revealed that the positive products of PCR amplication return to DHA-1 type for all 22 isolates (1 isolates of A. lwoffii and 21 isolates of A. baumannii) (figure, 3 and 4).

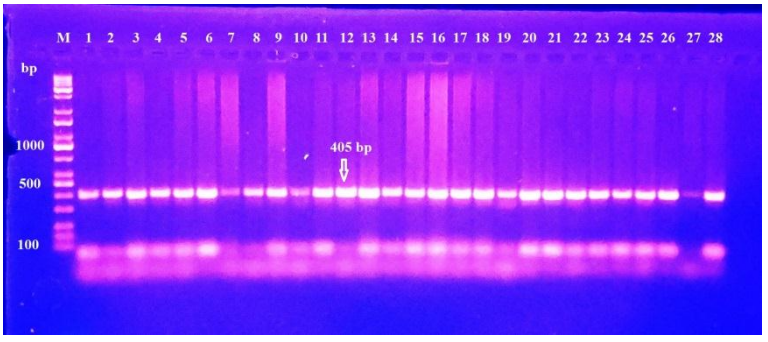


Figure (1): PCR amplification of DHA gene among *A. baumannii* isolates

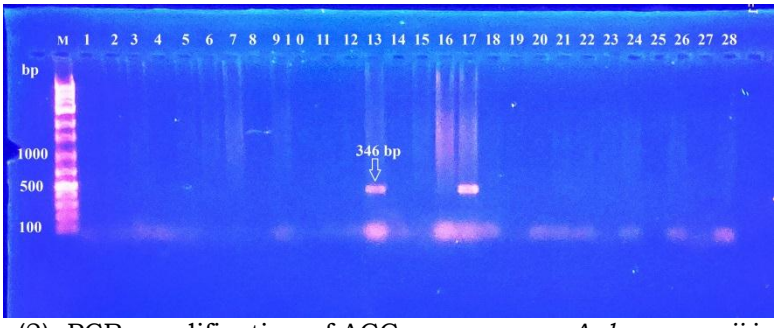


Figure (2): PCR amplification of ACC gene among *A. baumannii* isolates

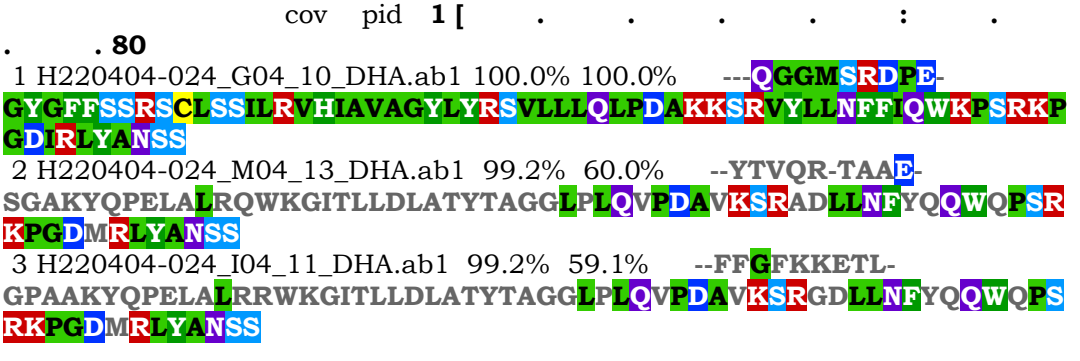
Score	Expect	Method	Identities	Positives	Gaps
208 bits(529)	3e-66	Compositional matrix adjust.	100/101(99%)	100/101(99%)	0/101(0%)

Query 25
WKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQWQPSRKPGDMRLYANSSIGLF
GA 84

WKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQWQPSRKPGDMRLYANSSIGLF
GA
Sbjct 120
WKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQWQPSRKPGDMRLYANSSIGLF
GA 179

Query 85 LTANAPGMPYEQLLTARILAPLGLSHTFITVPESAQSQYAY 125
LTANA GMPYEQLLTARILAPLGLSHTFITVPESAQSQYAY
Sbjct 180 LTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQYAY 220

Figure (3): Alignment of amino acid sequence for class C beta-lactamase DHA-1 gene between *A. baumannii* (no. 1) and *A. baumannii* strain (Sequence ID: EIJ3638142.1).



4 H220404-024_O02_6_DHA.ab1 100.0% 59.5% --
SLGLRVTSFKRYGAKYQPELLLRQWKGITLLDLATYTAGGLPLQVPDAVKSRADLL
NFYQQWQPSRKPGDMRLYANSS
5 H220404-024_C06_16_DHA.ab1 100.0% 60.3% --
HAGPKVGALKGPAANYQPELVLRQWKGITLLDLATYTAGGLPLQVPDAVKSRADL
LNFYQQWQPSRKPGDMRLYANSS
6 H220404-024_E06_17_DHA.ab1 98.4% 58.7% --TSLTE-
VALNDPAAKYQPELALPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQ
WQPSRKPGDMRLYANSS
7 H220404-024_E04_9_DHA.ab1 100.0% 61.1% --
DIGFKERSPEGSGANYQPELVLRQWKGITLLDLATYTAAGLWLQVPDAVKSRADLL
NFYQQWQPSRKPGDMRLYANSS
8 H220404-024_I06_19_DHA.ab1 99.2% 60.3% --NSWHEIRRP-
GYGANYQPELVLPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQWQPS
RKPGDMRLYANSS
9 H220404-024_I08_27_DHA.ab1 97.6% 60.8% ---V-F-VRSP-
GSGAKYQPELVLPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQWQPS
RKPGDMRLYANSS
10 H220404-024_A06_15_DHA.ab1 99.2% 61.1% --TMVHEVRRP-
GYGSNTSRELVLRQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQWQPS
RKPGDMRLYANSS
11 H220404-024_M02_5_DHA.ab1 100.0% 59.1% -
RGGVLARKAIKRTTQLTCKSYPLRQLKGITLLDLATYTAGGLPLQVPDAVKSRADL
LNFYQQWQPSRKPGDMRLYANSS
12 H220404-024_G02_2_DHA.ab1 99.2% 58.6% NRFGL-
VRKAIKRTEQNTSRELALPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQ
QWQPSRKPGDMRLYANSS
13 H220404-024_I02_3_DHA.ab1 97.6% 61.5% ------
VRKAIKRTEQNTSRELALPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQ
QWQPSRKPGDMRLYANSS
14 H220404-024_G06_18_DHA.ab1 98.4% 61.4% -QNG-A-
VRKAVKGTEHYQPQLALPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQ
QWQPSRKPGDMRLYANSS
15 H220404-024_O04_14_DHA.ab1 98.4% 60.6% -LID-E-
VRKALRVRSNTSRSLVLPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQ
WQPSRKPGDMRLYANSS
16 H220404-024_K06_20_DHA.ab1 98.4% 60.3% --QL--
GERKALRVRQQYQPELALPQWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFY
QQWQPSRKPGDMRLYANSS
17 H220404-024_K04_12_DHA.ab1 100.0% 65.6% --GFCGLRYEGV-
GPTQITSRSCFSAWKGITLLDLATYTAAGLPLQVPDAVKSRADLLNFYQQWQPSRK
PGDMRLYANSS
18 H220404-024_O06_22_DHA.ab1 100.0% 66.9% ---TLLRAYAKP-
GYGAITSRSCLSAWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQWQPSRK
PGDMRLYANSS
19 H220404-024_K02_4_DHA.ab1 99.2% 61.1% --IVV-
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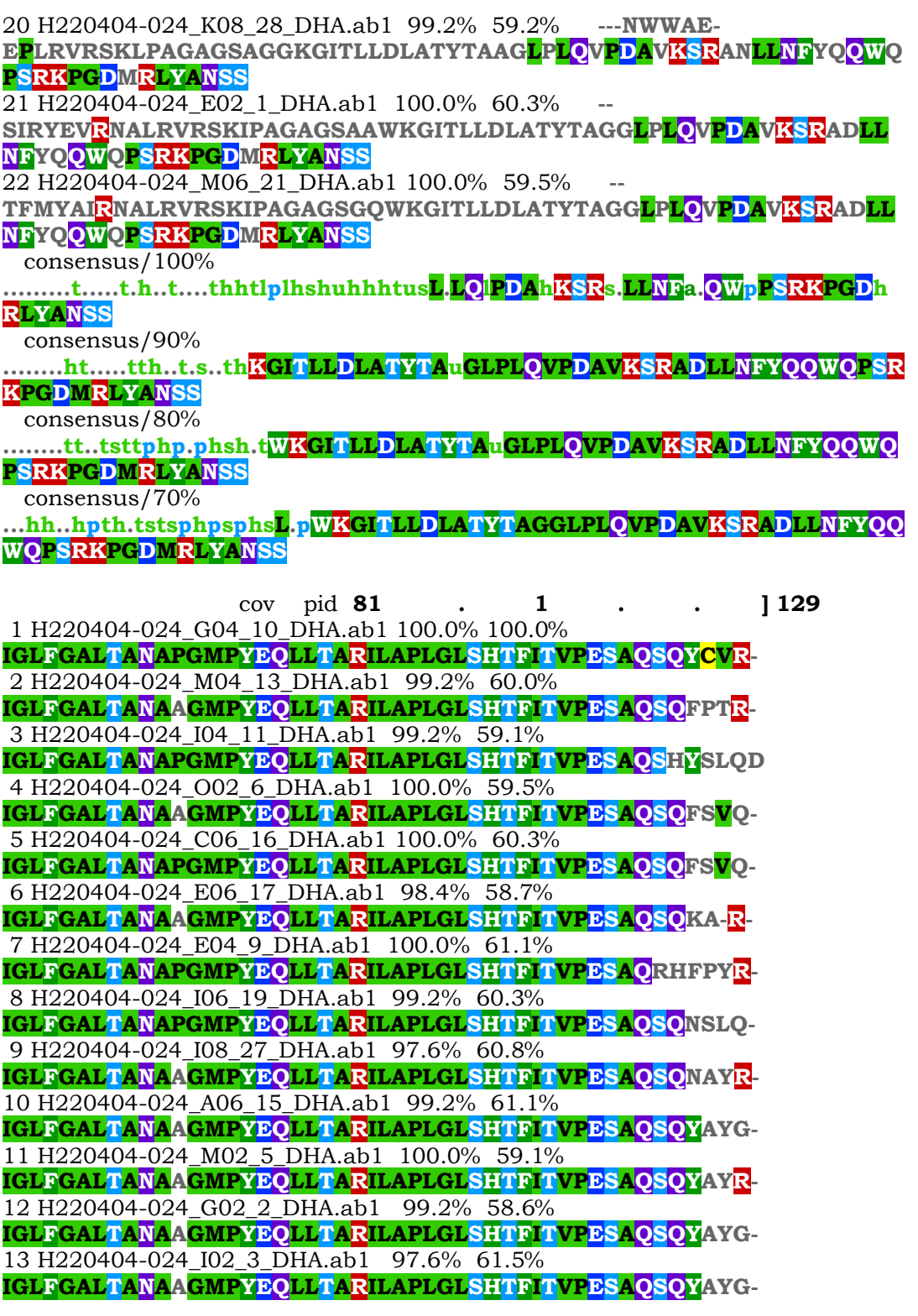
20 H220404-024_K08_28_DHA.ab1 99.2% 59.2% ---NWWAE-
EPLRVRSKLPAGAGSAGGKGITLLDLATYTAAGLPLQVPDAVKSRANLLNFYQQWQ
PSRKPGDMRLYANSS
21 H220404-024_E02_1_DHA.ab1 100.0% 60.3% --
SIRYEVRNALRVRSKIPAGAGSAAWKGITLLDLATYTAGGLPLQVPDAVKSRADLL
NFYQQWQPSRKPGDMRLYANSS
22 H220404-024_M06_21_DHA.ab1 100.0% 59.5% --
TFMYAIRNALRVRSKIPAGAGSGQWKGITLLDLATYTAGGLPLQVPDAVKSRADLL
NFYQQWQPSRKPGDMRLYANSS
consensus/100%
.....t.....t.h..t....thhtlplhshuhhhtusL.LQlPDAhKSRs.LLNFa.QWpPSRKPGDh
RLYANSS
consensus/90%
.....ht.....tth..t.s..thKGITLLDLATYTAuGLPLQVPDAVKSRADLLNFYQQWQPSR
KPGDMRLYANSS
consensus/80%
.....tt..tsttphp.phsh.tWKGITLLDLATYTAuGLPLQVPDAVKSRADLLNFYQQWQ
PSRKPGDMRLYANSS
consensus/70%
...hh..hpth.tstsphpsphsL.pWKGITLLDLATYTAGGLPLQVPDAVKSRADLLNFYQQ
WQPSRKPGDMRLYANSS
cov pid 81 . 1 . .] 129
1 H220404-024_G04_10_DHA.ab1 100.0% 100.0%
IGLFGALTANAPGMPYEQLLTARILAPLGLSHTFITVPESAQSQYCVR-
2 H220404-024_M04_13_DHA.ab1 99.2% 60.0%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQFPTR-
3 H220404-024_I04_11_DHA.ab1 99.2% 59.1%
IGLFGALTANAPGMPYEQLLTARILAPLGLSHTFITVPESAQSHYSLQD
4 H220404-024_O02_6_DHA.ab1 100.0% 59.5%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQFSVQ-
5 H220404-024_C06_16_DHA.ab1 100.0% 60.3%
IGLFGALTANAPGMPYEQLLTARILAPLGLSHTFITVPESAQSQFSVQ-
6 H220404-024_E06_17_DHA.ab1 98.4% 58.7%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQKA-R-
7 H220404-024_E04_9_DHA.ab1 100.0% 61.1%
IGLFGALTANAPGMPYEQLLTARILAPLGLSHTFITVPESAQRHFPYR-
8 H220404-024_I06_19_DHA.ab1 99.2% 60.3%
IGLFGALTANAPGMPYEQLLTARILAPLGLSHTFITVPESAQSQNSLQ-
9 H220404-024_I08_27_DHA.ab1 97.6% 60.8%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQNAYR-
10 H220404-024_A06_15_DHA.ab1 99.2% 61.1%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQYAYG-
11 H220404-024_M02_5_DHA.ab1 100.0% 59.1%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQYAYR-
12 H220404-024_G02_2_DHA.ab1 99.2% 58.6%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQYAYG-
13 H220404-024_I02_3_DHA.ab1 97.6% 61.5%
IGLFGALTANAAGMPYEQLLTARILAPLGLSHTFITVPESAQSQYAYG-

Figure (4): Parctial Alignment of amino acid sequence for class C beta-lactamase DHA-1 gene between 22 isolates of *A. baumannii* in current study

Discussion

Nosocomial infections caused by the Acinetobacter genus are increasingly linked to epidemics and have become a concern in almost any hospital in the world (Almasaudi, 2018). There have been numerous cases of nosocomial outbreaks of *A. baumannii* in the past decade, most commonly reported in the surgical, burn, and intensive care units (Jafari and Karbasizade, 2014).

Data of the present study was closer to the previous Locally study, according to Abdullah and Merza, (2019) they mention a total of 603 clinical samples of burn and sputum were collected from Duhok City, Iraq, 41 isolates of *A. baumannii* obtained. Another local study done by Mohammed *et al.*, (2022) found The spreading of *Acinetobacter* Species was detected in 9.2% of the states and the males were three times more likely to be infected with *Acinetobacter* than females.

These results are close nearer to previous studies obtained by Safwat et al., (2011) and Kateete *et al.*, (2017) who found the rates of *A. baumannii* isolates involved 3.8% and 5.4% which were isolated from different clinical sources. A molecular study done by Fekri *et al.*, (2017) The CIT gene was found in 39 samples (65%), the DHA gene in 36 samples (60%), the MOX gene in 12 samples

(20%), and the ACC and FOX genes were found in 8 samples (13.3%), according to multiplex PCR data for detection distribution AmpC-family genes among 60 isolates of *A. baumannii*.

A recent study was done by baserisalehi *et al.*, (2022) in Iran they observer that the distribution of AmpC-family genes among A. *baumannii* isolates were 27 (46%), 7 (12%), and 1(2%) for MOX, CIT and DHA genes respectively. Another molecular work done on 31 multidrug-resistant *A. baumannii* isolates by Yin *et al.*, (2008) they recorded rate of DHA gene was 3.2% according on methods of Pulse-field gel electrophoresis. Despite the fact that CMY is the most prevalent kind of AmpC-lactamase in the world, current work observed that DHA-1gene was exist in all isolates of *Acinetobacter* spp. and this finding may be closer to what it observed by Liu *et al.*, (2016) they mention that DHA-1 were the farthest common in China.

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