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Detection of Plasmid-mediated AmpC β-lactamases in Nosocomial Isolates of Gram-negative Bacilli

SAJID ABDELSADA SAHEL* AND INTIDHAAR NAEEM ABID

Department of Pathological Analysis, College of Science, University of Thi-Qar, Iraq
**(e-mail: sajidlion@sci.utq.edu.iq; Mobile :(+964)78107 38751)*

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ABSTRACT

Plasmid-mediated AmpC β-lactamases are uninducible in contrast to chromosomal AmpC, and they are frequently linked to widespread multidrug resistance. This study was aimed at detecting plasmid- mediated AmpC β-lactamases by phenotypic and genotypic methods in nosocomial Gram-negative isolates. Two hundred and fifty-four samples were collected from Al-Hussein and Al-Nasiriyah teaching hospitals in Al-Nasiriyah city, 194 from hospitalized patients (urine, burn swab surgical swab and sputum) and 60 samples from hospital environments (from medical staff, beds of patients, floor, ventilators, taps and operation hall). Out of 123 positive cultures of aerobic bacteria, 97 (78.86%) were Gram-negative bacilli; 89 (91.75%) from hospitalized patients and 8 (8.24%) from hospital environments. *E. coli* was the most common Gram-negative bacilli isolated. Carbapenems (imipenem and meropenem) were the most effective on nosocomial Gram-negative isolates. Out of 75 Gram-negative producing β-lactamase isolates; 52 (69.33%) were AmpC β-lactamase producers by cefoxitin susceptibility disc, while 28 (37.33%) by the AmpC disc test. The PCR product of plasmid-mediated AmpC β-lactamase (*bla*DHA and *bla*CIT) genes were detected in 8 (26%) and 10 (33.33%), respectively. *E. coli* was the most prevalence isolates causing nosocomial infections among Gram-negative bacilli. Carbapenems (imipenem and meropenem) effected on nosocomial Gram-negative isolates. *Klebsiella pneumonia* was the predominant isolates carrying plasmid-mediated AmpC β-lactamase genes.

Key words: Nosocomial infections, Gram-negative bacilli, AmpC, CIT, DHA

INTRODUCTION

Nosocomial infections are infections acquired in hospitals and these infections cross-between patients or between patients and hospital staff (Nasser *et al.*, 2019). Nosocomial infections (NIs) afflict hundreds of millions of patients worldwide and are one of the leading causes of death and morbidity in hospitals (Su *et al.*, 2021). Nosocomial infections are thought to affect more than two million patients each year in U. S. hospitals and have a significant influence on morbidity and mortality. Multidrug-resistant (MDR) bacteria have developed as a result of the overuse and abuse of antibiotics in both medicine and agriculture, and they are now understood to be a major contributor to nosocomial infections (Wang *et al.*, 2020). Infection with multidrug-resistant organisms may increase cost of management and the duration of hospital stay and may cause additional morbidity and mortality (Muter, 2019). A key contributor to the transmission of (MDR) bacteria are nosocomial infections (Jenkins, 2017). The formation of β-lactamases is one of the processes outlined above that is thought to be of the most significant since these enzymes are often transferrable and inactivate a variety of β-lactam antibiotics. AmpC β-lactamases belonged to group 1 cephalosporinases in Bush and Jacoby's classification scheme for β-lactamases based on functional features. There are two types of chromosomal or plasmid-mediated AmpC lactamases (Gebremichael *et al.*, 2020). AmpC cephalosporinases carried by plasmids were discovered in the late 1980s. The first plasmid-mediated DHA β-lactamase was discovered in a strain of *Salmonella* enteritidis in Dhahran (Saudi Arabia; Hennequin *et al.*, 2018). *Klebsiella pneumoniae* isolates harboring plasmid-mediated AmpC that have arisen through the transfer of chromosomal AmpC β-lactamase of *Morganella morganii* (Luk *et al.*, 2016). *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca* and *Proteus mirabilis* are the most common bacteria in which plasmid-mediated AmpC has been identified (Abdalhamid *et al.*, 2017). Most plasmid-based

AmpC genes are not inducible, but some of them, like the DHA-1 gene, are inducible by β-lactamase (Etemadi *et al.*, 2020).

MATERIALS AND METHODS

Two hundred and fifty-four samples were collected from different clinical specimens from hospitalized patients and beds, floors, knobs, ventilators, taps and medical staff in some Thi-Qar hospitals (Al-Hussein and Al-Nasiriyah teaching hospitals). The samples from patients include urine, burn swab, surgical swab and sputum. All the samples were transported to the laboratory and inoculated on blood agar and MacConkey agar and incubated at 37°C for 24 to 48 h. Identification of bacteria depended on morphological and microscopic examination, biochemical tests and confirmed by API 20 E and VITEK-2 systems.

Antimicrobial susceptibility of all the isolates were carried out against 17 different antibiotic disc by using Kirby-Bauer disc diffusion method (Parajuli *et al.*, 2017) as per CLSI guidelines (CLSI, 2020). for amoxicillin –clavulanate (20/10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), amikacin, (30 µg), aztreonam (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), tobramycin (10 µg), ampicillin (10 µg), cefepime (30 µg), trimethoprim (10 µg), nalidixic acid (10 µg), tetracycline (10 µg), meropenem (10 µg), ceftazidime (30 µg) and cefoxitin (30 µg). The rapid iodometric method was used to assess the production of β-lactamases in all isolates (Jameel and Amani, 2020).

Primary (screening) test for plasmid-mediated AmpC β-lactamases were carried out by using Cefoxitin disks (30 µg) to screen AmpC-producing isolates according to CLSI guidelines. Isolates with an inhibitory zone diameter measuring ≤18 mm were suspected of being AmpC β-lactamase producers (CLSI, 2020).

AmpC disc test was carried out to phenotypically identify the generation of AmpC β-lactamases. On a Muller-Hinton agar MHA plate, an *E. coli* ATCC 25922 lawn culture was grown. Several colonies of the test organism were injected onto a sterile disk (6 mm) that had been soaked with Tris-EDTA. The disc was then placed on the inoculated plate next to a cefoxitin disc, closely touching, with the inoculated side facing the agar. The plates were incubated at 37°C overnight. A flattening or indentation of the cefoxitin inhibition zone adjacent to the disc caused by the test strain was interpreted as a sign that AmpC β-lactamase was produced. A zone with no distortion was considered as negative (Shagufta *et al.*, 2017).

The DNA was isolated according to the manufacturer's instructions by using the Mini Kit (Favorgen, Austria). Polymerase chain reaction was performed using specific primers to detect plasmid-mediated AmpC β-lactamases (*bla*-DHA and *bla*-CITM) genes. By using multiplex PCR, the cefoxitin-resistant isolates were examined for the presence of plasmid-mediated AmpC genes. The primers used for PCR amplification and conditions are described in Tables 1 and 2, respectively. The PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide (Wassef *et al.*, 2014).

Table 2. Amplification conditions of genes used in this study (Wassef *et al.,* 2014)

Amplification conditions of DHA and CITM gene		
Initial denaturation	94°C	3 min
Denaturation 25 cycles	94°C	30 sec
Annealing	64°C	40 sec
Extension	72°C	1 min
Final extension	72°C	7 min

RESULTS AND DISCUSSION

Out of 254 samples collected during the study period, 123 (48.43) samples (109 hospitalized patients and 14 samples from the hospital environment) gave a positive result for culture, while 131 (51.57) (85 and 46 from patients and hospital environmental samples), respectively

Table 1. Primers used in this study (Wassef *et al.,* 2014)

S. No.	Primer product	Size		Sequence (5'-3')
1.	DHA	405bp	F	AACTTTCACAGGTGTGCTGGGT
			R	CCGTACGCATACTGGCTTTGC
2.	CITM	462bp	F	TGGCCAGAACTGACAGGCAAA
			R	TTTCTCCTGAACGTGGCTGGC

showed no bacterial growth (Table 3). The most common Gram-negative bacilli isolated during the current study was *E. coli* registering 35 (36.08%) among hospitalized patients followed by *K. pneumoniae* 24 (24.74%), *P. aeruginosa* 19 (19.59%), *A. baumannii* 6 (6.19%) and *E. cloacae* 5 (5.15%). On the other hand, *E. coli* was recorded highest number among Gram-negative bacteria from hospital environment isolates (Table 4).

The antibiotics resistance pattern of Gram-negative bacilli isolates in different clinical specimens against 17 antibiotics is presented in Fig. 1. The highest resistance level was recorded for ampicillin (90%), followed by trimethoprim (85%), amoxicillin with clavulanic acid (80%) and tetracycline (78%). The isolates showed resistance (70%) for 3GC cephalosporins (cefotaxime, cefepime, ceftazidime, ceftriaxone and cefotaxime) and moderate resistance rate for aztreonam and cefoxitin.

Enterobacteraces revealed lower resistance towards ciprofloxacin and chloramphenicol (25%). On the other hand, non-lactose fermenter exhibited higher percentage of

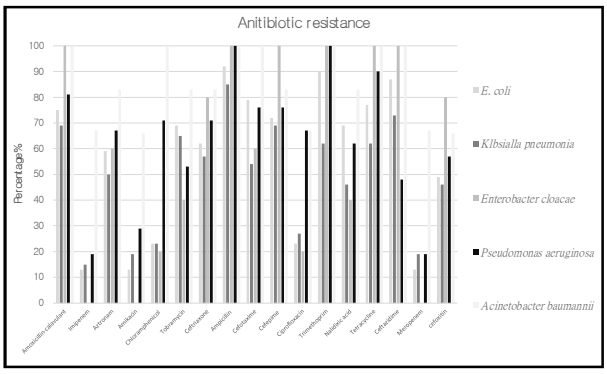

Fig. 1. Antibiotics resistance pattern of Gram-negative bacilli.

resistance (70%). Carbapenems (imipenem and meropenem) were the most effected on nosocomial Gram-negative isolates with resistance rate (20%) followed by amikacin (22%) except *A. baumannii* showing high resistance for carbapenems and amikacin. Total 97 isolates of Gram-negative bacilli were tested for their ability to the β-lactamase enzyme by the rapid iodometric method. The results found that 75 (77.32%) of isolates revealed positive results of β-lactamase production (Table 5).

Table 3. Types and numbers of specimens with positive results for bacterial culture isolated from different sources

Source of specimens		No. of specimens	No. and % of positive specimens	No. and % of negative specimens
Hospitalized patients		194	109 (88.62)	85 (64.89)
Hospital environment	Medical staff	10	2 (1.63)	8 (6.11)
	Beds of patients	10	4 (3.25)	6 (4.58)
	Floor	10	2 (1.63)	8 (6.11)
	Ventilators	10	1 (0.81)	9 (6.87)
	Taps	10	3 (2.44)	7 (5.34)
	Operation hall	10	2 (1.63)	8 (6.11)
	Total	254	123 (48.43)	131 (51.57)

Table 4. Types and numbers of Gram-negative bacteria isolated from different specimen hospitals

Source of specimens		Type of Gram-negative bacteria	No. and %	Total
Hospitalized patients				
		E. coli	35 (36.08)	89 (91.75%)
		K. pneumoniae	24 (24.74)	
		E. cloacae	5 (5.15)	
		P. aeruginosa	19 (19.59)	
		A. baumannii	6 (6.19)	
Hospital environment	Medical staff		0 (0.00)	8 (8.25%)
	Beds of patients	*E. coli*	1 (1.03)	
		K. pneumoniae	1 (1.03)	
	Floor	*E. coli*	2 (2.06)	
	Ventilators	*P. aeruginosa*	1 (1.03)	
	Taps	*E. coli*	1 (1.03)	
	Operation hall	*K. pneumoniae*	1 (1.03)	
		P. aeruginosa	1 (1.03)	

Table 5. Number and percentage of β-lactamase producing Gram-negative bacterial isolates by the rapid iodometric method

Source of sample	No. and % of β-lactamase producing isolates					Total
	E. coli (no.=39)	*K. pneumoniae* (no.=26)	*E. cloacae* (no.=5)	*P. aeruginosa* (no.=21)	*A. baumannii* (no.=6)	
Hospitalized patients	26 (89.66)	17 (89.47)	5 (100)	15 (88.24)	5 (100)	68 (90.6)
Hospital environment	3 (10.34)	2(10.53)	0 (0.00)	2 (11.76)	0 (0.00)	7 (9.4)
Total	29 (38.67)	19 (25.33)	5 (6.67)	17 (22.67)	5 (6.67)	75

Out of 75 Gram-negative producing β-lactamase isolates, 52 (69.33%) were AmpC β-lactamase producing cefoxitin susceptibility disc. The isolates were further confirmed by the AmpC disc test showing 28 (37.33%). The most common isolates of AmpC β-lactamase producers were *E. coli,* while *Enterobacter cloacae* were the lowest in percentage (Table 6). The PCR product of 30 AmpC β-lactamase producers showed that *bla*DHA gene detected 8 (26%) and *bla*CIT gene 10 (33.33%). *K. pneumonia* were the predominant isolates carrying AmpC β-lactamase genes (Table 7 and Figs. 2 and 3).

48.43% of results were generally culture-positive. Identifying the kind of illness is crucial for sample isolation since it may be a viral or fungal infection (Khan *et al.*, 2017). Recent study showed that *E. coli* were more frequent among Gram-negative isolates which caused nosocomial infections. *E. coli* are known to be responsible for a wide range of nosocomial infections, including gastroenteritis, septicemia, pneumonia, newborn meningitis and urinary tract infections (UTI; Jain *et al.*, 2021). On the other hand, *E. coli* isolates were the most common among other isolates from hospitalized patients. These results are similar to a previous study performed by Nouri *et al.* (2020)

Table 7. Numbers and percentages of plasmid-mediated AmpC β-lactamase genes

Bacteria	No. of isolates	No. and % isolates carry plasmid-mediated AmpC β-lactamase genes	
		CIT	DHA
E. coli	12	4 (40.00)	1 (12.50)
K. pneumonia	12	6 (60.00)	5 (62.50)
E. cloacae	2	0 (0.00)	1 (12.50)
P. aeruginosa	4	0 (0.00)	1 (12.50)
Total	30	10 (33.33)	8 (26)

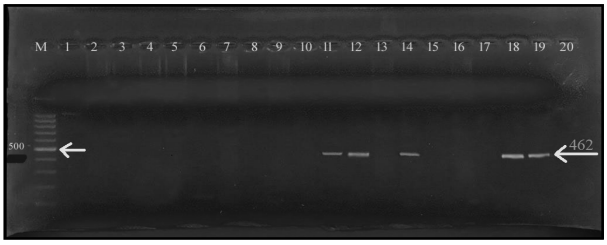

Fig. 2. Agarose gel electrophoresis of *bla*CIT gene (462) that showed the PCR product. M: Marker (2000-100bp), lanes (11,12,14,18,19) positive results of *bla*CIT gene and lanes (1-10,13,15-17, 20) negative results of *bla*CIT.

Table 6. Numbers and percentages of plasmid-mediated AmpC β-lactamase producing isolates that detection by phenotypic methods

Source of sample	Bacteria (no. of β-lactamase producer isolates)	Number and % plasmid-mediated AmpC β-lactamase producers isolates			
		Screening AmpC β-lactamase results		Confirmation AmpC β-lactamase results	
		Positive	Negative	AmpC disc method	
				Positive	Negative
Hospitalized patients	*E. coli* (26)	17 (32.69)	9 (39.13)	11 (39.29)	15 (31.91)
	K. pneumoniae (17)	12 (23.08)	5 (21.74)	8 (28.57)	9 (19.15)
	E. cloacae (5)	3 (5.77)	2 (8.70)	2 (7.14)	3 (6.38)
	P. aeruginosa (15)	11 (21.15)	4 (17.39)	4 (14.29)	11 (23.40)
	A. baumannii (5)	4 (7.69)	1 (4.35)	0 (0.00)	5 (10.64)
Hospital environment	*E. coli* (3)	2 (3.85)	1 (4.35)	1 (14.29)	2 (4.26)
	K. pneumoniae (2)	2 (3.85)	0(0.00)	2 (7.14)	0 (0.00)
	P. aeruginosa (2)	1 (1.92)	1 (4.35)	0 (0.00)	2 (4.26)
Total	75	52 (69.33)	23 (30.67)	28 (37.33)	47 (62.67)

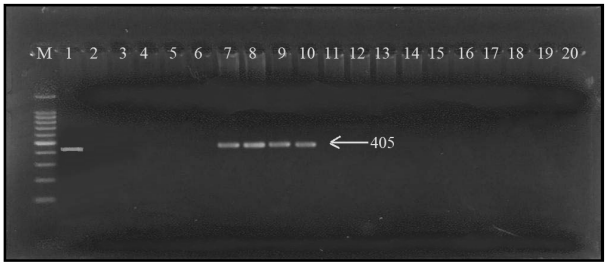

Fig. 3. Agarose gel electrophoresis of *bla*DHA gene (405) that showed the PCR product. M: Marker (2000-100bp), lanes (1,7-10) positive results of *bla*DHA gene and lanes (2-6,11-20) negative results of *bla*DHA gene.

in Hamadan, Iran, who showed that the most frequently isolated pathogen from all sites of infections was *E. coli*. These results disagree with Parajuli *et al.* (2017) who showed that *Acinetobacter baumannii* and *K. pneumoniae* were the most frequently isolated pathogens (Parajuli *et al.*, 2017). The results showed high resistance of non-lactose fermenters to most antibiotics compared to these lactose fermenters which may be due to organisms often do not respond to most cephalosporins, penicillins, or fluoroquinol-onees (Mehrad *et al.*, 2015). The results showed that all our isolates were resistant to the penicillin group (amoxicillin/clavulanic acid and ampicillin), the resistance of bacteria to this group of antibiotic may be due to changes in penicillin-binding proteins (PBPs) due to the fact that this group's activity depends on attaching to and deactivating membrane-bound penicillin-binding proteins (PBPs), which limit halt cell growth and the development of the cell wall (Jain *et al.,* 2021). A study by Teklu *et al.* (2019) and Jain *et al.* (2021) showed the high rate of resistance of Gram-negative bacteria of nosocomial isolates to ampicillin and amoxicillin with clavulanic acid. While Gram-negative bacteria isolated from nosocomial by Abebe et al. (2019) who found lower percentage of resistance to amoxicillin/ clavulanic acid (47%). Meropenem and imipenem were found to be the most active agents1for Gram-negative1bacteria. In the study, imipenem had shown broad spectrum activity1against1all selected1Gram-positive and Gram-negative bacterial isolates. These results are similar to those of Wang *et al.* (2020) who reported a high susceptibility rate to meropenem and imipenem.

The study showed that the most of Gram-negative isolates producing β-lactamase were *E. coli* followed by *K. pneumoniae* may be due to the high prevalence of these pathogens causing nosocomial infections and the most common harbor of β-lactamases (Khalifa *et al.*, 2021). Cefoxitin disc screening for AmpC has been recommended in earlier research, although it is non-specific (Abdel *et al.*, 2021). The results of this study showed that a high percentage of Gram-negative isolates was cefoxitin resistance, *E. coli* and1*K. pneumonia* were the most isolates that recorded positive results. These results are in agreement with previous studies conducted by Aryal *et al.* (2020) finding 50% of nosocomial Gram-negative bacilli isolates resistance to cefoxitin. This percentage was higher than that reported by Panwar and Chavan (2020) which was 23.2% for Gram-negative bacteria isolates from hospitalized patients.

AmpC disc test revealed that 37.33% of Gram-negative isolates were AmpC producers. These results are in agreement with the results of Ibrahim *et al.* (2019) who found 32.5% AmpC β-lactamase producers of Gram-negative nosocomial isolates. These results were higher than the results of Kolhapure *et al.* (2015) who recorded 10.33% isolates producing inducible AmpC β-lactamases by AmpC disc test. Phenotypic methods alone may not reflect the true number of PMABLs producers. Hence, molecular studies remain the gold standard for detection of plasmid AmpC β-lactamases (Shaimaa *et al.*, 2016). The most common plasmid-encoded AmpC enzymes are CIT and DHA. The current study revealed that1*K. pneumoniae* and *E. coli* were the isolates carrying bla_{CIT}, and this could be the fact that these genes were carried on the plasmid, and due to the large spread of Gram-negative bacteria, the acquisition of such genes was easy to isolates. These results agree with the previous study conducted by Ghonaim and Moaety (2018) and Fallah *et al.* (2020) who reported that bla_{CIT} gene was the most prevalent among *E. coli* and *K. pneumoniae* hospitalized patients isolates. The PCR results of this study showed that *K. pneumoniae* recorded a higher percentage of bla_{DHA}. The DHA enzyme is currently found worldwide, mainly in *Klebsiella* spp., but also to a lesser extent in *E. coli*, and other Gram-negative bacteria (Hennequin *et al.*, 2018). Previous studies agree with this finding (Ghanavati *et al.*, 2017; Kazemian *et al.*, 2019) who noticed that *K. pneumoniae* the

isolates carried these genes. In addition to their increased cephalosporin resistance, plasmid-mediated AmpC producing isolates may also have therapeutic significance because of the possibility of developing carbapenem resistance through further mutations that would limit porin production. In conclusion, *E. coli* was the most prevalence isolate causing nosocomial infections among Gram-negative bacilli. Carbapenems (imipenem and meropenem) had the most effect on nosocomial Gram-negative isolates. *K. pneumonia* was the predominant isolate which carried plasmid-mediated AmpC β-lactamase genes.

REFERENCES

Abdalhamid, B., Albunayan, S., Shaikh, A., Elhadi, N. and Aljindan, R. (2017). Prevalence study of plasmid-mediated AMPC β-lactamases in Enterobacteriaceae lacking inducible ampC from Saudi hospitals. *J. Med. Microbiol.* **66**: 1286-1290.

Abdel Aal, A., Khalil, N., Rashed, H. A., Abd-Elrahman, M. and El-Melegy, T. (2021). Genetic detection of AmpC beta-lactamase among gram negative isolates "A Single Center Experience". *Egyp. J. Immuno.* **2021**: 195-205. *https://doi.org/10.55133/eji.280120.*

Abebe, M., Tadesse, S., Meseret, G. and Derbie, A. (2019). Type of bacterial isolates and antimicrobial resistance profile from different clinical samples at a Referral Hospital, Northwest Ethiopia: Five years data analysis. *BMC Res. Notes* **12**: 01-06.

Aryal, S. C., Upreti, M. K., Sah, A. K., Ansari, M., Nepal, K., Dhungel, B., Adhikari, N., Lekhak, B. and Rijal, K. R. (2020). Plasmid-mediated AmpC β-lactamase CITM and DHAM genes among Gram-negative clinical isolates. *Inf. Drug Res.* **13**: 4249. *https://doi.org/10.2147/IDR.S284751.*

CLSI (2020). CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing, 30th edn. In: CLSI **40**: 001-293.

Etemadi, S., Ebrahimzadeh Leylabadlo, H. and Ghotaslou, R. (2020). AmpC β-lactamase among Enterobacteriaceae: A new insight. *Gene Reports* **19**: 100673. *https://doi.org/10.1016/j.genrep.2020.100673.*

Fallah, F., Azimi, T., Azimi, L., Karimi, A., Rahbar, M., Shirdoust, M., Sedighi, I., Saadat Sadeghi, A. and Armin, S. (2020). Evaluating the antimicrobial resistance and frequency of AmpC β-lactamases blaCMY-2 gene in Gram-negative bacteria isolates collected from selected hospitals of Iran: A multicenter retrospective study. *Gene Rep.* **21**: 100868. *https://doi.org/10.1016/j.genrep.2020.100868.*

Gebremichael, S., Id, T., Shiferaw Teklu, D., Tullu, K. D., Kinde Birru, S. and Legese, M. H. (2020). Extended-spectrum β-lactamase and AmpC beta-lactamases producing gram negative bacilli isolated from clinical specimens at International Clinical Laboratories, Addis Ababa, Ethiopia. *https://doi.org/10.1371/journal.pone.0241984.*

Ghanavati, R., Darban-Sarokhalil, D., Navab-Moghadam, F., Kazemian, H., Irajian, G. and Razavi, S. (2017). First report of coexistence of AmpC beta-lactamase genes in *Klebsiella pneumoniae* strains isolated from burn patients. *Acta Microbiol. et Immunologica Hungarica* **64**: 455-462.

Ghonaim, R. A. and Moaety, H. A. (2018). Comparison between multiplex PCR and phenotypic detection methods for identifying AmpC β-lactamases among clinical isolates of Enterobacteriaceae in Zagazig University Hospitals, Egypt. *Clin. Microbiol. Open Access* **7**: *https://doi.org/10.4172/2327-5073.1000313.*

Hennequin, C., Chlilek, A., Beyrouthy, R., Bonnet, R. and Robin, F. (2018). Diversity of DHA-1-encoding plasmids in *Klebsiella pneumoniae* isolates from 16 French hospitals. *J. Antimicrobial Chemotherapy* **73**: 2981-2989.

Hennequin, C., Ravet, V. and Robin, F. (2018). Plasmids carrying DHA-1 β-lactamases. *European J. Clin. Microbiol. Infect. Dis.* **37**: 1197-1209.

Ibrahim, M. E., Abbas, M., Al-Shahrai, A. M. and Elamin, B. K. (2019). Phenotypic characterization and antibiotic resistance patterns of extended-spectrum β-lactamase- and AmpC β-lactamase-producing Gram-negative bacteria in a referral hospital, Saudi Arabia. *Can. J. Inf. Dis. Med. Microbiol.* **2019**. *https://doi.org/10.1155/2019/6054694.*

Jain, N., Jansone, I., Obidenova, T., Simanis, R., Anis Meisters, J., Straupmane, D., Reinis, A. and Bagattini, M. (2021). Antimicrobial resistance in nosocomial isolates of Gram-negative bacteria: Public health implications in the Latvian context. *https://doi.org/10.3390/antibiotics10070791.*

Jameel, H. M. S. A. S. and Amani, M. A. (2020). Emergence of extended spectrum β-lactamases (ESBL) in clinical isolates of *Klebsiella pneumoniae* In: *Rada'a Thamar Cities, Yemen. Albaydha Univ. J.* **2** : 091-108. *https://doi.org/10.56807/BUJ.V2I2. 42.*

Jenkins, D. R. (2017). Nosocomial infections and infection control. *Medicine (United Kingdom)* **45**: 629-633. *https://doi.org/10.1016/j.mpmed.2017.07.005.*

Kazemian, H., Heidari, H., Ghanavati, R., Ghafourian, S., Yazdani, F., Sadeghifard, N., Valadbeigi, H., Maleki, A. and Pakzad, I. (2019). Phenotypic and genotypic characterization of ESBL-, AmpC- and carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates. *Med. Prin. Pract.* **28**: 547-551. *https://doi.org/10.1159/000500311.*

Khalifa, S. M., Abd El-Aziz, A. M., Hassan, R. and Abdelmegeed, E. S. (2021). β-lactam resistance associated with β-lactamase production and porin alteration in clinical isolates of *E. coli* and *K. pneumoniae*. *PLoS ONE* **16**: *https://doi.org/10.1371/JOURNAL.PONE.0251594.*

Khan, H. A., Baig, F. K. and Mehboob, R. (2017). Nosocomial infections: Epidemiology, prevention, control and surveillance. *Asian Pacific J. Trop. Biomed.* **7**: 478-482.

Kolhapure, R., Kumar, A. and Rajkumar, H. (2015). Coexpression of ESBL, Amp C and MBL in gram negative bacilli. *Int. J. Res. Med. Sci.* **3**: 2698-2703.

Luk, S., Wong, W. K., Ho, A. Y., Yu, K. C., To, W. K, and Ng, T. K. (2016). Clinical features and molecular epidemiology of plasmid-mediated DHA-type AmpC β-lactamase-producing *Klebsiella pneumoniae* blood culture isolates, Hong Kong. *J. Glob. Antimicrob. Res.* **7**: 37-42.

Mehrad, B., Clark, N. M., Zhanel, G. G. and Lynch, J. P. (2015). Antimicrobial resistance in hospital-acquired gram-negative bacterial infections. *Chest* **147**: 1413-1421.

Muter, B. A. (2019). Bacteriological study of diabetic foot ulcer and it's relation to metabolic control in AL- Nassriah city /Iraq. *Univ. Thi-Qar J. Sci.* **3**. *https://doi.org/10.32792/utq/utjsci/vol3/2/6.*

Nasser, N. E., Abbas, A. T. and Science, C. (2019). Bacterial contamination and its susceptibility in intensive care unit in Thi-qar province/Iraq. *Univ. Thi-Qar J. Sci.* **3**. *https://doi.org/10.32792/utq/utjsci/vol3/4/3.*

Nouri, F., Karami, P., Zarei, O., Kosari, F., Alikhani, M. Y., Zandkarimi, E., Zarandi, E. R. and Taheri, M. (2020). Prevalence of common nosocomial infections and evaluation of antibiotic resistance patterns in patients with secondary infections in Hamadan, Iran. *Inf. Drug Res.* **13**: 2365-2374.

Panwar, S. D. and Chavan, S. K. (2020). Identification and antibiogram of Amp C β-lactamases present in gram negative bacilli – Has the trend changed? *Ind. J. Microbiol. Res.* **7**: 182-191.

Parajuli, N. P., Acharya, S. P., Mishra, S. K., Parajuli, K., Rijal, B. P. and Pokhrel, B. M. (2017). High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. *Antimicrob. Res Inf. Cont.* **6**: 01-09.

Santiago, G. S., Gonçalves, D., da Silva Coelho, I., de Mattos de Oliveira Coelho, S. and Neto Ferreira, H. (2020). Conjugative plasmidic AmpC detected in *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* human clinical isolates from Portugal. *Brazil. J. Microbiol.* **51**: 1807-1812.

Shagufta, R., Fomda, B., Gulnaz, B., Lubna, S., Jan, A., Mohd, S. and Junaid, A. (2017). Prevalence of AmpC β-lactamase in Gram negative bacilli by different phenotypic methods in a Tertiary Care Institute in Kashmir. *Brit. J. Med. Med. Res.* **19**: 01-09.

Shaimaa, A. S., W. A. G., El-Shafii, S. and Farag, A. (2016). Occurrence of plasmid-mediated ampC β-lactamases (PMABLs) resistance genes among *E. coli* O157:H7 isolated from bovine diarrhea. *Egyp. J. Chem. Environ. Health* **2**: 45-55.

Su, C., Zhang, Z., Zhao, X., Peng, H., Hong, Y., Huang, L., Huang, J., Yan, X., Wu, S. and Bai, Z. (2021). Changes in prevalence of nosocomial infection pre- and post-COVID-19 pandemic from a tertiary hospital in China. *BMC Inf. Dis.* **21**: 01-07.

Teklu, D. S., Negeri, A. A., Legese, M. H., Bedada, T. L., Woldemariam, H. K. and Tullu, K. D. (2019). Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. *Antimicrob. Res. Inf. Cont.* **8**: *https://doi.org/10.1186/S13756-019-0488-4.*

Wang, Q., Wang, Z., Zhang, F., Zhao, C., Yang, B., Sun, Z., Mei, Y., Zhao, F., Liao, K., Guo, D., Xu, X., Sun, H., Hu, Z., Chu, Y., Li, Y., Ji, P. and Wang, H. (2020). Long-term continuous antimicrobial resistance surveillance among nosocomial gram-negative bacilli in China from 2010 to 2018 (CMSS). *Inf. Drug Res.* **13**: 2617-2629.

Wassef, M., Behiry, I., Younan, M., Guindy, N. El, Mostafa, S. and Abada, E. (2014). Genotypic identification of AmpC β-lactamases production in gram-negative bacilli isolates. *Jundishapur J. Microbiol.* **7**: 01-08.