Determination of antimicrobial resistance profiles and molecular detection of carbapenemases in Gram negative bacilli isolated from different sources in Mosul city, Iraq

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Abstract

The objectives of the present study were to determine the antimicrobial resistance profiles and evaluate the occurrence of metallo-β-lactamase and other carbapenemases in Gram-negative bacilli isolated from different sources in Mosul city. Bacterial isolates were recovered from clinical, veterinary, and environmental specimens and samples. Pure isolates were identified and tested for determination of their antimicrobial resistance profiles using disk diffusion method. Phenotypic detection of metallo-carbapenemase-producing bacteria was conducted using combined disk method. Imipenem-resistant strains were subjected to molecular detection of carbapenemase genes (*bla* $_{VIM}$, *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{OXA-48}$, *bla* $_{IMP}$) using multiplex PCR. Three hundred and ninety three bacterial isolates were recovered from (365) specimens and samples, 246 isolates (62.6%) were multi-drug resistant (MDR). The isolates were highly resistant to amoxicillin-clavulanic acid, tetracycline, cefotaxime, sulfamethoxazole, and ceftriaxone (96.7%, 65.1%, 64.1% and 63.6%, and 63.1% respectively). Carbapenems were the most effective antimicrobials used, the percentages of isolates resistant to imipenem and meropenem were (12.5%), and (10.7%), respectively. The study found that (7.4%) of the isolates were metallo-carbapenemase producers, phenotypically. Multiplex PCR results revealed that 47/49 (95.5%) imipenem-resistant isolates were positive for PCR detection test, isolates with double genes (*bla* $_{KPC}$ + *bla* $_{VIM}$ and *bla* $_{KPC}$ + *bla* $_{NDM}$) were the most prevalent (n=9; 18.4% for each), followed by *bla* $_{KPC}$ and *bla* $_{NDM}$ (n=8; 16.3% for each), and *bla* $_{VIM}$ (n=6; 12.2%). Three isolates (6.1%) were positive for *bla* $_{IMP}$ and two others (4.1%) were positive for each of *bla* $_{OXA-48}$ and *bla* $_{KPC}$ + *bla* $_{OXA-48}$, while two isolates (4.1%) gave a negative result for the test. In conclusion, carbapenemase genes were detected in the environmental isolates as well as in the clinical and veterinary ones, which might suggest the transmission of carbapenemase-producing bacteria and /or resistance determinants from clinical and veterinary settings to the environment.

Keywords: Carbapenemase genes; gram-negative bacilli; metallo-carbapenemase; Mosul (Iraq); multiplex PCR

1. Introduction

Carbapenems are the most powerful antimicrobial agents used nowadays for treating bacterial infections (Jean *at al.*, 2015; Livermore & Woodford 2000). Unfortunately, with the extensive use of these drugs, the occurrence of carbapenems-resistant Gram negative bacilli (GNB) has increased significantly representing a serious challenge facing the use of antimicrobials in all aspects (Ali *et al.,* 2021; Jean *at al.*, 2015; Nordmann *et al*., 2011). However, carbapenemase production is considered as the most prevalent mechanism used by the resistant bacteria to overcome the cidal action of carbapenems (Queenan & Bush 2007).

β-lactamases are classified based on the functional classification into four groups (1-4), or according to Ambler molecular classification into four classes (A-D) (Bush and Jacoby 2010; Ambler *at al*., 1980). Carbapenemases are currently located within the functional groups 2d, 2f, 3, and according to the molecular classification their types fall within the classes A, B, and D (Jean *at al.*, 2015; Queenan & Bush 2007; Ambler *at al*., 1980). The emergence of carbapenemases in Gram-negative bacilli is rapidly increasing especially during the past years (Loqman *et al*., 2021; Karabay *et al*., 2016), consequently these enzymes reduce the effectiveness of carbapenems which are the last resort against severe bacterial infections that are resistant to treatment in hospitals and various health institutions (Jean *at al.*, 2015; Nordmann *et al*., 2011; Queenan & Bush 2007).

Class A carbapenemases are one of the serine β-lactamases characterized by their sensitivity to clavulanic acid and other traditional β-lactamase inhibitors, they include: Guiana Extended Spectrum enzymes (GES), *S. marcescens* enzymes (SME), Imipenem- hydrolyzing β-lactamase (IMI), *K.pneumoniae* Carbapenemases (KPC), and Non-Metallo Carbapenemases (NMC) (Jean *at al.*, 2015; Queenan & Bush 2007).

Class B carbapenemases are metallo enzymes with expanded capabilities to degrade almost all β-lactams without being affected by traditional β-lactamase inhibitors (Bush and Jacoby 2010). They have zinc divalent cations in their active sites crucial for the enzymatic activity (Bush and Jacoby 2010; Ambler *at al*., 1980). Metallo-carbapenemases are usually inhibited by chelator compounds such as EDTA. Among metallo β-lactamases, Verone integron mediated metallo enzymes (VIM), Imipenemase (IMPs), and New Delhi metallo enzymes (NDM) are the most prevalence, whereas Sao Paulo metallo enzymes (SPM), Seoul Imipenemase (SIM), German Imipenemase (GIM) are nearly endemic (Bush and Jacoby 2010; Queenan & Bush 2007; Ambler *at al*., 1980).

Class D carbapenemases (also known as OXA enzymes) are specifically present in the MDR- *Acinetobacter baumannii* and have been responsible for many hospital's outbreaks associated with high mortality rates. Isolates of GNB other than *A. baumannii* carrying $bla_{\text{OXA-48}}$ genes were also reported (Loqman *et al*., 2021; Carrër *et al*., 2008). These enzymes are distinguished by their lack of inhibition by both clavulanic acid and EDTA (Bush & Jacoby 2010; Queenan & Bush 2007). Among the most important enzymatic types of this group are: OXA-48, OXA-58, OXA-23, and OXA-24 (Bush & Jacoby 2010).

The occurrence and global spread of carbapenemase-producing bacteria (CPB) represent an urgent concern worldwide, with the growing sources for these resistant strains not only in the health and veterinary settings but even in the environment and the community as well (Guerra *et al*., 2014; Nordmann *et al*., 2011; Queenan & Bush 2007). The real prevalence of CPB in Mosul city is not well understood. Therefore, it is essential to conduct a surveillance studies investigating the occurrence of CPB in Mosul and use the collected data in the formulation of control measures that assist in the prevention of their spread. Since the detection of β-lactamases, including carbapenemases, represents the first most important step in evaluating their dissemination and prevalence (Elbadawi *et al*., 2021; Al-Hasso & Khalaf 2020; Cui *et al*., 2019), the present work aimed to screen different bacterial species isolated from various sources in Mosul city for the presence of metallo-β-lactamases (class B) and determine the prevalent carbapenemase genes.

2. Materials and Methods

The study was conducted in Mosul city, Iraq for the period from April 2019 to October 2020. The study included 365 specimens and samples (clinical, veterinary, and environmental). The clinical specimens (n=120) were varied between pus, sputum, urine, and diarrhea taken from patients who visited several Mosul hospitals, while the veterinary specimens (n=135) represented by rectal swabs of infected chicken with diarrhea from poultry farms in Mosul. Environmental samples (n=110) were taken from different sources (soil and water bodies) and different sites of the city. The study and data accumulation were carried out with the approval of Biophysics Dept. Board No. 28c on 27/3/2019.

2.1. Bacterial Isolation and Identification

For the isolation and the identification of bacteria, all specimens and samples were treated with the standard bacteriological methods. Briefly, the samples were grown on special culture media, which included MacConkey agar, blood agar, and nutrient agar (Himedia Co., India). The pure isolates were subjected to microscopic examination after staining with Gram's stain, in addition to biochemical tests (IMViC, oxidase, nitrate reduction, DNase, carbohydrate fermentation, TSI, gelatin liquefaction, phenylalanine deaminase, and urease). Bacterial isolates were identified presumptively based on their morphological shapes, cultural characteristics, and biochemical tests (Procop *et al*., 2017; MacFaddin 1980).

2.2. Antimicrobial Susceptibility Testing (AST)

AST was conducted for all bacterial isolates against different groups of antimicrobials (Bioanalyse Co., Turkey) using Kirby-Bauer disc diffusion method. Briefly, Mueller-Hinton agar plates were inoculated with fresh bacterial inoculums equivalent to 0.5 McFarland standards and incubated at 35^{o}C for 16-18 hrs. after applying antimicrobial disks. The results were interpreted based on CLSI recommendations (CLSI 2021). Standard strains *P. aeruginosa* ATCC 27853 and *E.coli* ATCC 25922 were used as qualitative control. Bacterial

isolates resistant to three or more different classes of antimicrobials were considered as Multi-Drug Resistant strains (CLSI 2021; Thapa *et al*., 2017).

2.3. Phenotypic Detection of Metallo-Carbapenemase-Producing Strains

A suspension of the fresh bacterial culture equivalent in turbidity to 0.5 McFarland standard tube was used, the suspension was diluted (1:10), and inoculated on a Mueller-Hinton Agar plate. Two discs of meropenem, one with 10 µl of (0.5 M) EDTA and another without EDTA, were placed on the medium (25mm apart). An increase in the diameter of the inhibition zone equal to 7 mm or more in the case of the EDTA-containing disc compared with the other disc was considered as a positive result of the screening test (Thapa *et al*., 2017; Franklin *et al*., 2006).

2.4. Molecular Detection of Carbapenemase-Producing Strains

Carbapenem resistant isolates were molecularly tested for the presence of carbapenemase genes (*bla*$_{VIM}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$) using multiplex PCR technique. Preparation of DNA template was performed according to Presto Mini gDNA Bacteria Kit from Geneaid Biotech Ltd. (www.geneaid.com). Molecular detection was conducted using 96-well thermal cycler Optimus 96G, England. The reaction mixture (50 µl) composed of (25 µl) master mix (Bioneer co.) and (1 µl) DNA template. The concentrations of each of the forward and reverse primers used in a single PCR tube were as follows: 15 pmoles/µl for *bla*$_{KPC}$, *bla*$_{IMP}$, and *bla*$_{VIM}$; 20 pmoles/µl for *bla*$_{NDM}$; and 25 pmoles/µl for *bla*$_{OXA-48}$. The PCR program performed was as follows; an initial denaturation step at 95°C for 5 min, then 35 cycles of DNA denaturation at 95°C for 45 s, and primer annealing at 60°C for 45 s. Primer extension was at 72°C for 1 min, and final extension at 72°C for 8 min (Amer *et al*., 2016; Doyle *et al*., 2012). PCR products were examined by agarose gel electrophoresis with 1.5% agarose in 0.5x Tris-borate-EDTA (TBE) buffer. Gene bands were visualized and confirmed with the aid of a UV transilluminator. Table (I) summarizes the amplicon sizes and the primer sequences used in the study.

Table 1. Primers used for the detection of carbapenemase genes.

Carbapenemase gene	Amplicon size (bp)	Primer sequences*
*bla*KPC	900	5'-TGTCACTGTATCGCCGTC-3' 5'-CTCAGTGCTCTACAGAAAACC-3'
*bla*IMP	587	5'-GAAGGCGTTTATGTTCATAC-3' 5'-GTACGTTTCAAGAGTGATGC-3'
*bla*VIM	389	5'-GTTTGGTCGCATATCGCAAC-3' 5'-AATGCGCAGCACCAGGATAG-3'
*bla*NDM	782	5'-GCAGCTTGTCGGCCATGCGGGC-3' 5'-GGTCGCGAAGCTGAGCACCGCAT-3'
*bla*OXA-48-like	438	5'-GCGTGGTTAAGGATGAACAC-3' 5'-CATCAAGTTCAACCCAACCG-3'

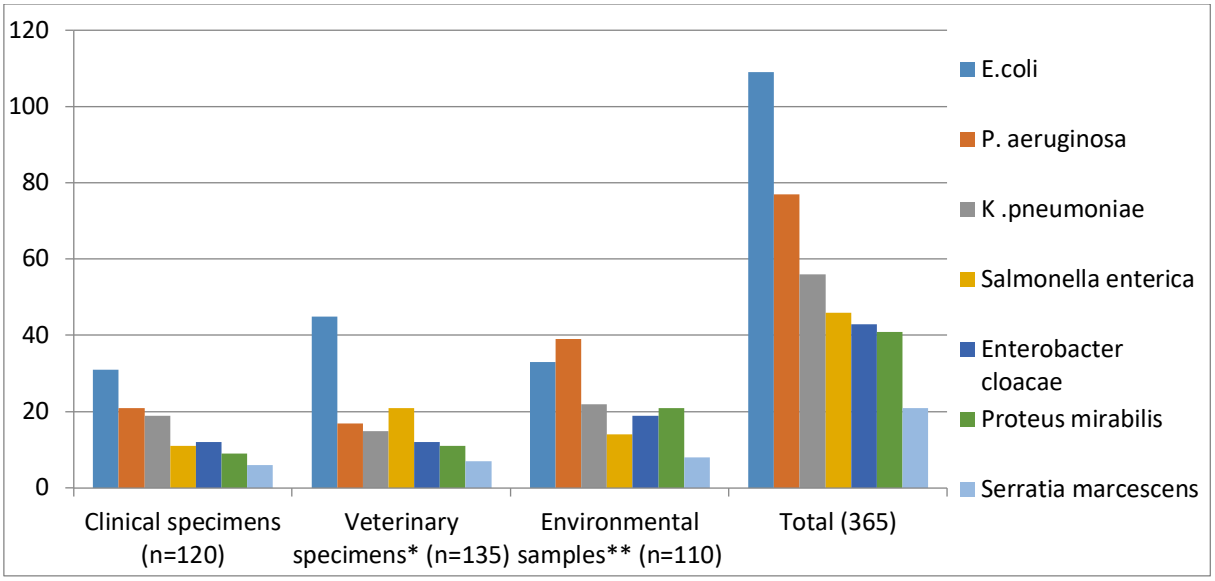
* The first and second primers for each gene are forward and reverse primers, respectively.

2.5. Statistical Analysis

Data was statistically analyzed using one-way ANOVA (SPSS software ver. 21) for the comparison of categorical data, p-values of ≤ 0.05 and ≤ 0.01 were considered as significant and highly significant, respectively. Additionally, percentages were used for the expression of antimicrobial resistance profiles and phenotypic metallo-β-lactamase detection results.

3. Results

From the total of 365 specimens and samples collected in the study, 393 bacterial strains were isolated (Figure 1). The isolation rates varied according to the bacterial species and the source of the isolation. *E.coli* isolates had the highest percentage (n=109, 27.7%), while *Serratia marcescens* was the lowest (n=21, 5.4%). The number and percentage of bacteria that were isolated from environmental samples were the highest (n=156, 39.7%) with a highly significant difference at p-value ≤ 0.01 in comparison to that isolated from clinical specimens (n=109, 27.7%). On the other hand, the number of bacteria isolated from veterinary specimens (n=128, 32.6%) has a significant difference at p-value ≤ 0.05 in comparison to clinical specimens. There was no significant difference between bacterial numbers isolated from veterinary specimens and environmental samples (Figure 1).



* Significant difference between total bacterial number isolated from veterinary and clinical specimens at p≤ 0.05.
** Highly significant difference between total bacterial number isolated from environmental samples and clinical specimens at p≤ 0.01.

Fig. 1. Number and sources of bacterial isolates used in the study.

Of the total 393 bacterial isolates recovered in the study, 380 (96.7%) were resistant to amoxicillin-clavulanic acid, which proved to be ineffective against the vast majority of the studied isolates. The percentage of resistance to ceftazidime, cefotaxime and ceftriaxone was (57%), (64.1%) and (63.1%) respectively (Table 3). The isolates showed high resistance to tetracycline and sulfamethoxazole (65.1% and 63.6%, respectively), their resistance to

ciprofloxacin was (20.6%). Carbapenems were the most effective antimicrobials used, the percentages of isolates resistant to imipenem and meropenem were (12.5%), and (10.7%), respectively (Table 2). The results showed that 246 out of 393 isolates (62.6%) were Multi-Drug Resistant (MDR) where *P. aeruginosa*, *S.enterica* , and *K.pneumoniae* isolates represented the most MDR strains. The most antimicrobial-resistant isolates were from clinical specimens followed by veterinary specimens, while isolates from environmental samples were the least resistant to antimicrobials (Figure 2).

Table 2. Number and percentage (%) of antimicrobial resistant isolates.

Bacterial species	Antimicrobials tested *										
	CTX	CAZ	IPM	MEM	CRO	AMC	FOX	CIP	TET	SXT	GEN
E.coli (n=109)	60(55)	49(45)	13(11.9)	10(9.2)	59(54.1)	99(90.8)	68(62.4)	22(20.2)	68(62.4)	61(56)	45(4.3)
P. aeruginosa (n=77)	59(76.6)	52(67.5)	18(23.4)	15(19.5)	49(63.6)	77(100)	67(87)	26(33.8)	63(81.8)	63(81.8)	65(84.4)
K .pneumoniae (n=56)	47(83.3)	47(83.9)	10(17.9)	9(16.1)	(80.3)45	56(100)	38(67.9)	17(30.6)	47(83.9)	(80.3)45	39(69.6)
S. enterica (n=46)	33(71.7)	30(65.2)	8(17.4)	8(17.4)	39(84.8)	46(100)	29(63)	8(17.4)	36(78.3)	37(80.4)	27(58.7)
E. cloacae (n=43)	6(60.5)2	20(46.5)	0(0)	0(0)	26(60.5)	43(100)	14(32.6)	3(7)	15(34.9)	17(39.5)	6(14)
P. mirabilis (n=41)	10(24.4)	9(22)	0(0)	0(0)	15(36.6)	39(95.1)	(51.2) 21	3(7.3)	16(39)	18(43.9)	6(14.6)
S. marcescens (n=21)	17(81)	17(81)	0(0)	0(0)	15(71.4)	20(95.2)	11(52.4)	2(9.5)	11(52.4)	9(42.8)	3(14.3)
Total (n=393)	252(64.1)	224(57)	49(12.5)	42(10.7)	248(63.1)	380(96.7)	248(63.1)	81(20.6)	256(65.1)	250(63.6)	191(48.6)

* CXT: cefotaxime, CAZ: ceftazidime, IPM: imipenem, MEM: meropenem, CRO: ceftriaxone, AMC: amoxicillin-clavulanic acid , FOX: cefoxitin, CIP: ciprofloxacin, TET: tetracycline, SXT: sulfamethoxazole, GEN: gentamicin

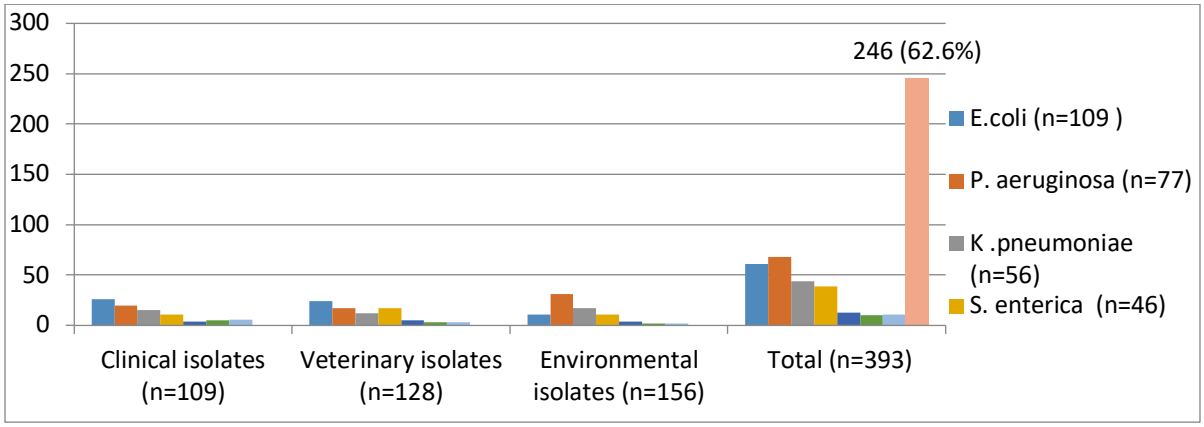


Fig. 2. Number, percentage (%), and sources of Multi-Drug Resistant (MDR) isolates.

Among the 393 bacterial isolates of Gram-negative bacilli recovered in the study, 29 isolates (7.4%) gave a positive result for the detection test of metallo-carbapenemase production according to the compound disc method used (Figure 3). *P. aeruginosa* isolates were the most producing species compared to the rest of the isolates (n=14, 18.2%), followed by *E.coli*, *K. pneumoniae* and *Salmonella enterica* (7.3%, 7.1%, and 6.5%, respectively) (Figure 4). It is noteworthy that 13 isolates out of 109 (11.9%) were from clinical specimens, 10/128 isolates (7.8%) were from veterinary specimens, and 6/156 isolates (3.8%) were from environmental samples (Figure 4).

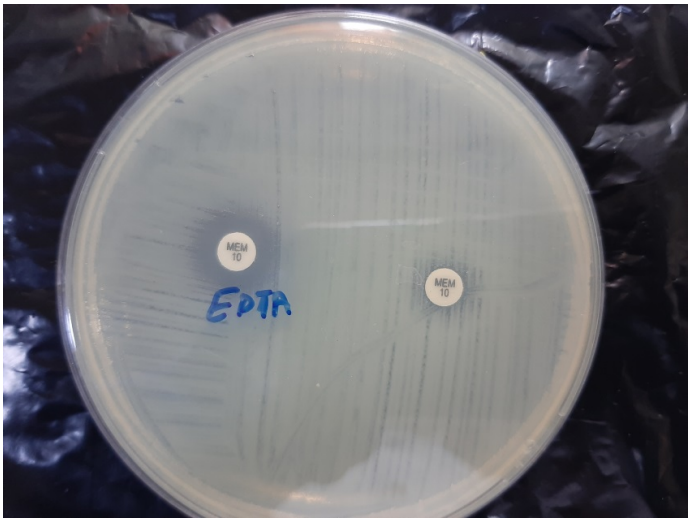


Fig. 3. Phenotypic metallo-carbapenemase detection by combined disk method.

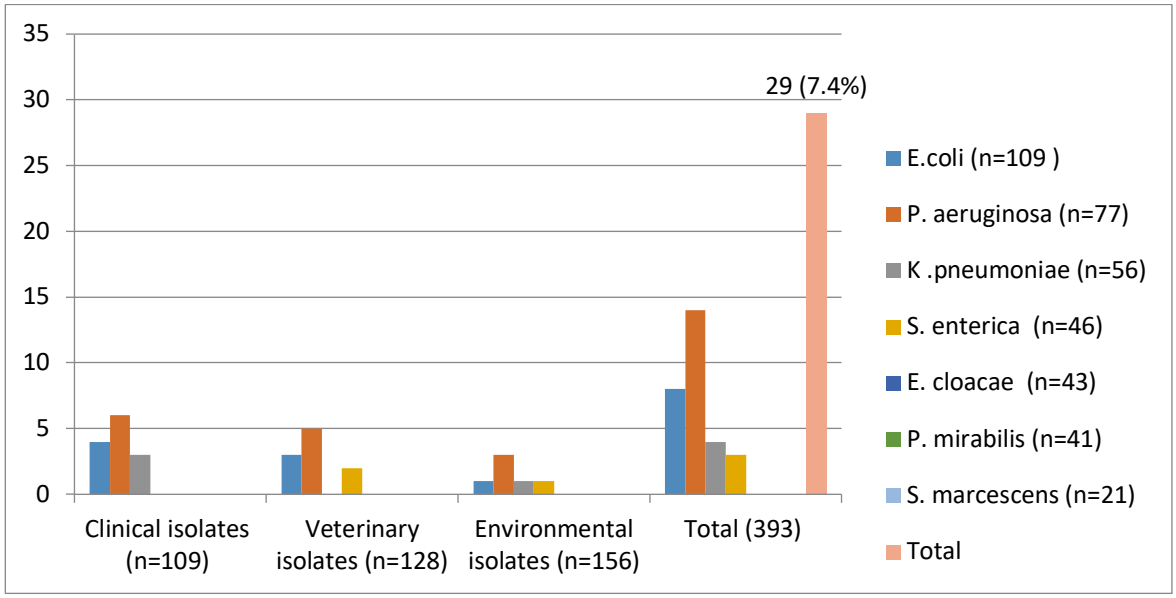


Fig. 4. Number and percentage (%) of phenotypically metallo-carbapenemase positive isolates.

Out of 49 imipenem resistant isolates, 47 isolates (95.5%) were positive for the molecular detection by multiplex PCR. The most prevalent genes detected were *bla*$_{KPC}$ + *bla*$_{VIM}$ and *bla*$_{KPC}$ + *bla*$_{NDM}$ (n=9; 18.4% for each), followed by *bla*$_{KPC}$ and *bla*$_{NDM}$ (n=8; 16.3% for each), and *bla*$_{VIM}$ (n=6; 12.2%). Three isolates (6.1%) were positive for *bla*$_{IMP}$ and two others (4.1%) were positive for each of *bla*$_{OXA-48}$ and *bla*$_{KPC}$ + *bla*$_{OXA-48}$, while two isolates (4.1%) gave a negative result for the PCR molecular detection test (Table 3).

Table 3. Distribution of carbapenemase genes among bacterial isolates.

Carbapenemase gene	*P. aeruginosa* (n=18)			*E.coli* (n=13)			*K .pneumoniae* (n= 10)			*S. enterica* (n=8)			Total (n= 49)
	C*	V	E	C	V	E	C	V	E	C	V	E	
bla$_{KPC}$	2			2	1	1	-		2	-			8(16.3%)
bla$_{IMP}$	2			1			-			-			3(6.1%)
bla$_{VIM}$	2	1	1	-			1			1			6(12.2%)
bla$_{NDM}$	1	1		-	1		-	1		1	1	2	8(16.3%)
bla$_{OXA-48-like}$	-			1			1			-			2(4.1%)
bla$_{KPC}$ + *bla*$_{VIM}$	2	2	1	1			1		1	1			9 (18.4%)
bla$_{KPC}$ + *bla*$_{NDM}$	3			1	1		-	2		1		1	9 (18.4%)
bla$_{KPC}$ + *bla*$_{OXA-48-like}$	-			1			1			-			2 (4.1%)
PCR negative	-					2	-			-			2(4.1%)
Total genes detected													**47 (95.9%)**

* C: clinical isolates ; V: veterinary isolates ; E: environmental isolates

4. Discussion

In this study (393) Gram-negative bacilli were isolated from (365) clinical, veterinary, and environmental specimens and samples. The isolated species included: *K. pneumoniae, E.coli*, *P. aeruginosa*, *S. marcescens, S. enterica*, *E. cloacae*, and *P. mirabilis* with various isolation rates (Figure 1). The isolates showed variable antimicrobial resistance patterns ranged between high resistance rates to amoxicillin-clavulanic acid, tetracycline, cefotaxime and sulfamethoxazole (96.7%, 65.1%, 64.1% and 63.6% respectively) and low resistance rates to ciprofloxacin, imipenem and meropenem (20.6%, 12.5% and 10.7%, respectively as shown in Table 2). Our results revealed that (246) isolates (62.6%) were MDR strains (Figure 2). These findings are lower than the results reported by Thapa *et al*. (2017) in their study conducted on clinical Gram negative bacteria isolated in Nepal where resistance rates to cefotaxime, ciprofloxacin, ceftazidime, imipenem, sulfamethoxazole and gentamicin were 89%, 76%, 43.9%, 8.3%, 72% and 65% respectively. Loqman *et al*. (2021) also reported, in their study conducted in Morocco on clinical *Enterobacteriaceae* isolates, higher rates in comparison to our results (ciprofloxacin 87%, sulfamethoxazole 87.8% and gentamicin 100%). In China, Zhang *et al*. (2018) found that 26.8%, 36.6% and 43.9% of carbapenem-resistant clinical *K .pneumoniae* isolates were resistant to ciprofloxacin, sulfamethoxazole, and gentamicin, respectively and these results were lower than ours (30.6%, 80.3% and

69.6%, respectively), however their resistance rates to ceftazidime and ceftriaxone were 100% for each antibiotic compared to 83.9% and 80.3% reported in the present study. Our results were close to the findings of an Iranian study (Rahbar *et al*., 2008) as they reported 40% and 30% resistance rates for *K. pneumoniae* and *P. aeruginosa* clinical isolates to ciprofloxacin (we reported 30.6% and 33.8%, respectively), as well they reported 80% and 73% resistance rates to ceftazidime, respectively for both species in comparison to 83.9% and 67.5% in our study. In the study of Loqman *et al*. (2021) they reported the occurrence of carbapenems resistance in 131/1603 (8.2%) clinical strains of *Enterobacteriaceae* and this result was close to ours (Table 2). As for MDR *P. aeruginosa* and *K. pneumoniae*, our results were higher (88.3% and 78.6% respectively) in comparison to (44.4% and 38.7%, respectively) that reported by Thapa *et al*. (2017) but similar for MDR *E.coli* (56%) and lower than MDR *Enterobacter* and *P. mirabilis* (30.2% and 24.4%, respectively). Our results showed that 62.6% of the isolates were MDR, as 246 isolates out of 393 were resistant to three basic groups of antimicrobials (clinical isolates gave the largest rate (79.8%), followed by veterinary (63.3%), and environmental (50%) isolates as illustrated in figure 2. This indicates the occurrence and the prevalence of MDR strains even in the environmental samples which are supposed to be low in rates, this may be attributed to the misuse, irresponsible, and often unmonitored use of antimicrobials, specifically β-lactams, as discussed later.

As illustrated in figure 4, among 393 isolates recovered in the present study, only 29 isolates (7.4%) were found to be metallo-carbapenemase producing bacteria by EDTA Combined Disk (CD) method. The positive isolates include *P.aeruginosa* (n=14), *E.coli* (n=8), *K.pneumoniae* (n=4), and *S.enterica* (n=3). Thirteen of them were isolated from clinical specimens, 10 were isolated from veterinary specimens while the other six were from environmental samples (Figure 4). Our results were close to the findings of Thapa *et al*. (2017) who reported a detection rate of 5.8% among 362 Gram negative isolates, but higher than that reported by Mishra *et al*. (2012) which was 1.3% of GNB isolated from lower respiratory tract infections in Nepal and that reported by Al-Charrakh *et al*. (2016) which was 5.3% of *P.aeruginosa* isolates recovered from clinical specimens in Baghdad (Iraq). On the other hand, our findings were lower than that reported by Amer *et al*. (2016) which was 19.1% of clinical *Enterobacteriaceae* isolated in Egypt, and Jamal *et al*. (2020) in their study conducted in Iraq as they found that (17.1%) of *K.pneumoniae* clinical isolates were positive for metallo-β-lactamases (MBLs) detection test. Elbadawi *et al*. (2021) also reported a phenotypic detection rate for (MBLs) of 50.9% among Carbapenem-Resistant *Enterobacteriaceae* (CRE) isolated from hospitalized patients in Sudan, while Loqman *et al*. (2021) found that 25.9% of CRE were positive for metallo- carbapenemase CD detection test. Also, Anoar *et al*. (2014) reported in their study conducted in Sulaimani city (Iraq) that 46/177 isolates (25.9%) of the clinical Gram negative bacteria tested were MBLs positive. Moreover, in the studies of Bhat *et al.* (2013) in India and Hussein *et al*. (2018) in Wasit city (Iraq) they found that (37% and 31%, respectively) of *Pseudomonas* clinical isolates were

MBLs positive by EDTA double-disc test, these result are almost double than ours as we reported 18.2% of *P. aeruginosa* isolates with positive detection test (Figure 4).

Molecular detection of carbapenemase genes by multiplex PCR revealed that 20 isolates out of 49 tested (40.8%) have double genes (*bla*$_{KPC}$ + *bla*$_{VIM}$ 9/49, *bla*$_{KPC}$ + *bla*$_{NDM}$ 9/49, *bla*$_{KPC}$ + *bla*$_{OXA-48}$- 2/49). As single genes, *bla*$_{KPC}$ and *bla*$_{NDM}$ were the most prevalent genes (8/49, 16.3% for each) followed by *bla*$_{VIM}$ (6/49, 12.2%). The total detection rate was 95.9% as two isolates were negative for the detection test (Table 3). These findings are in consistence with the results of Amer *et al*. (2016) and Kazi *et al*. (2015) who reported a detection rate of carbapenemase genes of 97.7% and 98.2% respectively, but higher than the results of Elbadawi *et al*. (2021) and Jamal *et al.* (2020) who reported a molecular detection rate of 58.7% and 50%, respectively with *bla*$_{NDM}$ being the most prevalent gene in comparison to other genes. The gene *bla*$_{KPC}$ was most frequently detected in *P. aeruginosa* isolates followed by *E.coli*, *K. pneumoniae*, and *S. enterica* isolates, this finding was in contrast to what Amer *et al*. (2016) found in their study as they reported the occurrence of *bla*$_{KPC}$ in *K. pneumoniae* more frequently in comparison to other species. This may be attributed to the differences in the number of isolates and source of isolation in both studies. Interestingly, carbapenemase genes were detected in environmental isolates as well as in clinical and veterinary ones, which might suggest the existence of interplay between the three ecologies (clinical, veterinary, and environmental) in the transmission of resistant strains.

The results of the present study indicate the occurrence and the dissemination of CPB among bacterial isolates recovered from various specimens and samples in Mosul city. These findings represent a preliminary evidence of the existence of a relationship between the different sources of isolation. This is probably attributed to the wide use of antimicrobials in medical and veterinary aspects which contributes, as a selective factor, in accelerating the emergence of resistant strains possessing these enzymes (Ali *et al*., 2022; Bonardi & Pitino 2019; Zurfluh *et al*., 2017; Bhat *et al*., 2013). Furthermore, the resistance genes mediated by transmissible elements such as plasmids, transposons, and integrons play a great role in increasing the frequency of dissemination of these enzymes through horizontal genetic transfer between bacterial species (Ali *et al*., 2022; Le Terrier *et al*., 2020; Kazi *et al*., 2015).

The presence of CPB among environmental strains as well as among clinical and veterinary ones may suggest the possibility of clonal or strain dissemination from other sources which might be hospitals, or veterinary farms in which antimicrobials are used a lot whether as a therapy, a prophylaxis or as a growth promotors (Le Terrier *et al*., 2020; Bonardi & Pitino 2019; Zurfluh *et al*., 2017; Hamza *et al*., 2016). The resistant strains as a consequence of the selective pressure imposed by these drugs will flourish and dominate in these establishments and reach the environment through the effluent water, wastewater, discharged patients and poultry products. Thus, the environment will become an emergence source for the dissemination of these strains added to the existing ones (Hamza *et al*., 2016; EFSA 2013 ; Pesapane *et al*., 2013). The similarity between the environmental and the clinical CPB has been reported by Khan *et al*. (2018) who suggested the dispersion of these

strains from hospitals to aquatic environment and the probability of their presence in the community.

The detection of carbapenemase genes in 95.5% of the carbapenem resistant strains raises the alarm when taking into account the fact that carbapenems are the most effective and powerful agents currently available for treating bacterial infections and they represent our last line of defense in the face of resistant microorganisms. These results also shed light on the fact that such mechanism used by bacteria to resist carbapenems are present within the arsenal of local isolates in Mosul city, including environmental isolates and it is undoubtedly increasing with the continued use of carbapenems in hospitals and other health institutions in Mosul city recently.

5. Conclusion

The current study aimed primarily to detect the occurrence and the prevalence of carbapenemase genes in Gram-negative bacilli isolated from clinical, veterinary, and environmental sources in Mosul city. The results showed the presence of these enzymes in the environmental strains as well as in the clinical and veterinary ones, which indicates the emergence and the spread of such strains in the city's environments as well, rendering them to a new source for the dissemination of carbapenemase producing bacteria in the future with the growing use of carbapenems in the city. The outputs of the study concentrate mainly on the necessity of taking measures to control and monitor carbapenems usage in order to prevent the dissemination of CPB from health and veterinary settings to the environment.

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