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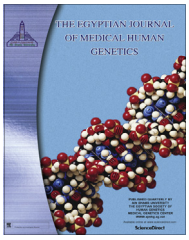

ORIGINAL ARTICLE

Occurrence and detection of AmpC β-lactamases among *Enterobacteriaceae* isolates from patients at Ain Shams University Hospital

Soha A. El-Hady *, **Lamiaa A. Adel** [1]

Microbiology & Immunology Department, Faculty of Medicine – Ain Shams University, Egypt

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KEYWORDS

AmpC β-lactamase;
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Multiplex PCR

Abstract *Background:* AmpC β-lactamases are often plasmid mediated that hydrolyze all β-lactam antibiotics except cefepime and carbapenems.

Aim of the study: We aimed to evaluate the presence of AmpC β-lactamase among *Enterobacteriaceae* isolates separated from patients with nosocomial infections, and to detect the genetic basis for AmpC production in these strains.

Subjects and methods: 50 AmpC β-lactamase *Enterobacteriaceae* were analyzed for the presence of AmpC production. Three phenotypic AmpC confirmation assays (AmpC E test, the disk approximation test, Amp C EDTA disc) were able to detect the majority of AmpC-positive strains correctly. Molecular detection of plasmid mediated AmpC by multiplex PCR was conducted on them.

Results: The results show that from the 148 total isolates obtained from ICU admitted patients with suspected nosocomial infections, 50 (33.8%) were AmpC β-lactamase isolates. For the 50 AmpC isolates, all phenotypic Amp C tests gave a positive result. Among these isolates, plasmid encoded AmpC genes were detected by multiplex PCR in 46 (92%) isolates, which included *Klebsiella pneumoniae* (n = 22) (47.8%), *Escherichia coli* (n = 15) (32.6%), and *Proteus mirabilis* (n = 9) (19.6%). One *E. coli,* one *K. pneumoniae*, and two *P. mirabilis* isolates showed no AmpC gene. The most prevalent AmpC gene was that belonging to family CMY-1 which was detected in 73.9% (34/46) of all isolates.

Conclusion: It could be concluded that: Amp C producing isolates among *Enterobacteriaceae* strains have been increasingly recognized in the Ain Shams University Hospital.

Thus, molecular identification of the genes encoding AmpC would be essential for a reliable epidemiological investigation of their transmission in hospitals.

1. Introduction

The prevalence of multidrug-resistant Gram-negative bacteria has increased continuously over the past few years, and

* Corresponding author. Tel.: +20 01221519480.
E-mail addresses: sohaelhady@yahoo.com (S.A. El-Hady), lamiaa3r@yahoo.com (L.A. Adel).
[1] Tel.: +20 01006885427.

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bacterial strains producing AmpC β-lactamases and/or extended spectrum β-lactamases (ESBLs) are of particular concern [14].

AmpC β-lactamases are clinically significant because they may confer resistance to penicillins, cephalosporins, oxyimino-cephalosporins (e.g., ceftriaxone, cefotaxime, and ceftazidime), cephamycins (e.g., cefoxitin and cefotetan), and monobactams. AmpC β-lactamase activity is not affected by the ESBL inhibitor clavulanic acid [25].

In the Ambler structural classification of β-lactamases AmpC enzymes belong to class C [3], while in the functional classification scheme of Bush et al., they were assigned to group 1 [5].

Genes for AmpC β-lactamases are commonly found on the chromosomes of several members of the family *Enterobacteriaceae*, including *Enterobacter*, *Shigella*, *Providencia*, *Citrobacter freundii*, *Morganella morganii*, *Serratia marcescens*, and *Escherichia coli* [8].

In Gram-negative bacteria, AmpC β-lactamase production is chromosome or plasmid mediated. Chromosomal AmpC genes are expressed constitutively at a low level. Some *Enterobacteriaceae*, such as *Enterobacter* species, *Citrobacter* spp., and *Serratia* spp., carry an inducible AmpC gene. In these cases, the gene is strongly induced by β-lactams, such as cefoxitin and imipenem, with expression mediated by the regulator AmpR. Mutations in the repressor gene AmpD may lead to overproduction of AmpC β-lactamases [14].

Plasmid-based AmpC genes are expressed constitutively in most cases. However, some plasmid-carried AmpC genes, such as the DHA-1 gene, are inducible by β-lactams, with expression regulated similar to that of inducible chromosomal AmpC genes. All plasmid-carried AmpC genes are considered to be of significant clinical relevance [20]. AmpC overproduction in addition to porin mutations of the outer membrane can reduce susceptibility to carbapenems, in particular in plasmid-mediated AmpC producers. Porins are chemically selective and transport only one group of molecules, or may be specific for one molecule. β-lactam and fluoroquinolone antibiotics must pass through porins to reach their targets in gram negative bacteria. Bacteria can develop resistance to these antibiotics by mutating the gene that encodes the porin – the antibiotics are then excluded from passing through the outer membrane [17].

Although reported with increasing frequency in case isolates, the true rate of occurrence of plasmid mediated AmpC β-lactamases in *Klebsiella pneumoniae*, *E. coli*, and *Proteus mirabilis* remains unknown. Many laboratories have difficulty in detecting these enzymes in clinical isolates [8].

There are no Clinical Laboratory Standards Institute (CLSI) or other approved criteria for AmpC detection. Organisms producing enough AmpC β-lactamase will typically give a positive ESBL screening test but fail in the confirmatory tests involving increased sensitivity with clavulanic acid [22]. This phenotype is not, however, specific for an AmpC producer, since it can occur with certain complex TEM mutants, OXA-type ESBLs, and carbapenemases and in strains with high levels of TEM-1 β-lactamase. Other confirmatory tests are needed [9].

This study aimed to evaluate the presence of AmpC β-lactamase among *Enterobacteriaceae* isolates separated from patients with nosocomial infections, and to detect the genetic basis for AmpC production in these strains.

2. Material and methods

This study was conducted on 50 AmpC β-lactamase *Enterobacteriaceae* isolates (23 *K. pneumoniae*, 16 *E. coli*, 11 *P. mirabilis*) in the Medical Microbiology and Immunology department of the Ain Shams University from a total of 148 *Enterobacteriaceae* clinical isolates, from intensive care unit (ICU) admitted patients attending the department of surgery of the Ain Shams University Hospital. Samples were collected in the period from May to October 2014. These isolates were obtained from various clinical specimens (55 respiratory specimens of sputum and endotracheal aspirate (ETA), 39 pus specimens from cases of open wounds or open abscesses using a sterile swab, and 29 mid-stream urine (MSU) taken from non catheterized patients and catheter-stream urine (CSU) in catheterized patients taken from the catheter tube using a sterile syringe, and 25 blood samples for blood culture). All samples were collected under aseptic conditions.

All isolates were cultured on Mac Conkey's agar (Difco/BD Diagnostics Systems, Sparks, MI, USA), blood agar media (Oxoid Ltd., Basingstoke, UK), and Cysteine Lactose Electrolytes Deficient (CLED) agar using 1 μl calibrated loop to detect colony forming unit (CFU) per milliliter (for urine specimens) and then incubated aerobically at 37 °C for 24 h. All isolated *Enterobacteriaceae* were worked up microbiologically and identified by conventional microbiological methods (colonial morphology, Gram staining, and biochemical tests) according to Cheesebrough [6].

Antibiotic susceptibility testing was performed using susceptibility test disks (Becton Dickinson, Germany), and interpretation was done according to 2013 CLSI guidelines [7]. Susceptibility testing was performed on Mueller–Hinton agar (bioMérieux, France), using overnight cultures at a 0.5 McFarland standard followed by incubation at 35 °C for 16–18 h.

2.1. AmpC screening using the disk diffusion method

Cefoxitin resistance strains were detected using cefoxitin disk 30-ug (Becton Dickinson). Isolates with zone diameters less than 18 mm were considered AmpC positive and selected for confirmation of AmpC production.

The phenotypic AmpC confirmatory tests were performed on all isolates by the AmpC E test (AB bio-Mérieux, Sweden), the disk approximation test, and Amp C EDTA disc. Then molecular detection of plasmid mediated AmpC by multiplex PCR was conducted on the 50 AmpC β-lactamase *Enterobacteriaceae* isolates.

2.2. Detection of AmpC β-lactamases

2.2.1. Phenotypic AmpC confirmation methods

1. AmpC E testing: The AmpC E test (AB bio-Mérieux, Sweden) for cefotetan susceptibility was performed according to the manufacturer's instructions. The AmpC E test consists of a strip containing cefotetan on one end and cefotetan-cloxacillin on the other end. Ratios of the MICs of cefotetan and cefotetan-cloxacillin of $\geqslant 8$ are considered positive for AmpC β-lactamase production.

2. Disk approximation test: The disk approximation test was designed to detect AmpC production. A conventional disk diffusion susceptibility assay is carried out with a susceptible strain, such as *E. coli* ATCC 25922. A 0.5 McFarland bacterial suspension from an overnight blood agar plate, Inoculate surface of the MHA plate. A 30 μg ceftazidime disk was placed at the center of the plate then 10 μg imipenem, 30 μg cefoxitin, and 20/10 μg amoxicillin/clavulanate disks were placed at a distance of 20 mm from the ceftazidime disk. The plate was inverted and incubated overnight at 37 °C. After overnight incubation, if there is any obvious blunting or flattening of the zone of inhibition between the ceftazidime disk and the inducing substrates (imipenem, cefoxitin and amoxicillin/clavulanate disk) it will be considered as a positive result for AmpC production [12].
3. AmpC EDTA disk test. The test is based on the use of Tris–EDTA to permeabilize a bacterial cell and release β-lactamases into the external environment. AmpC disks (i.e., filter paper disks containing Tris–EDTA) were prepared by applying 20 μl of a 1:1 mixture of saline and Tris–EDTA to sterile filter paper disks, allowing the disks to dry, and storing them at 2 to 8 °C. The surface of a Mueller–Hinton agar plate (Oxoid Ltd., Basingstoke, and Hampshire, England) was inoculated with a lawn of cefoxitin- susceptible *E. coli* ATCC 25,922 according to the standard disk diffusion method [4]. Immediately prior to use, AmpC disks were rehydrated with 20 μl of saline and several colonies of each test organism were applied to a disk. A 30 μg cefoxitin disk (Becton Dickinson, Sparks, MD) was placed at the inoculated surface of the Mueller–Hinton agar. The inoculated AmpC disk was then placed almost touching the antibiotic disk with the inoculated disk face in contact with the agar surface. The plate was then inverted and incubated overnight at 35 °C in ambient air. After incubation, plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result).

Molecular detection of plasmid mediated AmpC by multiplex PCR was conducted on the 50 AmpC β-lactamase *Enterobacteriaceae* isolates as follows:

2.2.2. Molecular detection of AmpC β-lactamase

2.2.2.1. Preparation of template DNA. A single colony of each organism was inoculated from a blood agar plate into 5 ml of broth and incubated for 20 h at 37 °C with shaking. Cells from 1.5 ml of the overnight culture were harvested by centrifugation for 5 min. After the supernatant was decanted, the pellet was resuspended in 500 μl of distilled water. The cells were lysed by heating at 95 °C for 10 min, and cellular debris was removed by centrifugation for 5 min. The supernatant, 2 μl (1/250 volume) of the total sample, was used as the source of template for amplification.

2.2.2.2. Protocol for multiplex. Multiplex PCR was performed for amplification of genes for the most common types of plasmid mediated AmpC β lactamases using the method described by Pérez-Pérez and Hanson [21]. PCR was performed in a DNA thermal cycler (Biometra, Germany) with a final volume of 50 μl in 0.5-ml thin-walled tubes. For the detection of CMY-1 gene 5-GCT GCT CAA GGA GCA CAG GAT-3 was used as forward primer (corresponding to nucleotides 358–378) and CAC ATT GAC ATA GGT GTG GTG C-3 was used as the reverse primer (corresponding to nucleotides 877–856) expected amplicon size 520 bp. For CMY-2 gene 5-TGG CCA GAA CTG ACA GGC AAA-3 was used as a forward primer (corresponding to nucleotides 478–498) and 5-TTT TC CTG AAC GTG GCT GGC-3 was used as a reverse primer (corresponding to nucleotides 939–919) expected amplicon size 462 bp. For ACC-1 gene 5-AAC AGC CTC AGC AGC CGG TTA-3 (corresponding to nucleotides 861–881) and 5-TTC GCC GCA ATC ATC CCT AGC-3 was used as a reverse primer (corresponding to nucleotides 1206–1186) expected amplicon size 346 bp. For FOX-1 gene 5-AAC ATG GGG TAT CAG GGA GAT G-3 was used as a forward primer (corresponding to nucleotides 1475–11496) and 5-CAA AGC GCG TAA CCG GAT TGG-3 was used as a reverse primer (corresponding to nucleotides 1664–1644) expected amplicon size 190 bp [21].

PCR was performed with a final volume of 50 μl in 0.5-ml thin-walled tubes. Each reaction contained 20 mM Tris–HCl (pH 8.4); 50 mM KCl; 0.2 mM each deoxynucleoside triphosphate; 1.5 mM $MgCl_2$; 0.6 μM primers and 1.25 U of *Taq* DNA polymerase (Life Technologies, Rockville, MD).

Template DNA (2 μl) was added to 48 μl of the master mixture and then overlaid with mineral oil. The PCR program consisted of an initial denaturation step at 94 °C for 3 min, followed by 25 cycles of DNA denaturation at 94 °C for 30 s, primer annealing at 64 °C for 30 s, and primer extension at 72 °C for 1 min. After the last cycle, a final extension step at 72 °C for 7 min was added. Five-microliter aliquots of PCR product were analyzed by gel electrophoresis with 2% agarose (Bio-Rad, Hercules, and Calif.). Gels were stained with ethidium bromide at 10 μg/ml and visualized by UV transillumination. A 100-bp DNA ladder from Life Technologies was used as a marker. Negative controls were PCR mixtures with the addition of water in place of template DNA.

Ideally, all the primer pairs in a multiplex PCR should enable similar amplification efficiencies for their respective target. This may be achieved through the utilization of primers with nearly identical optimum annealing temperatures (primer length of 18–30 bp or more and a GC content of 35–60% may prove satisfactory) and should not display significant homology either internally or to one another.

2.3. Statistical methods

Data were coded and entered using the statistical package SPSS version 15. Data were summarized using descriptive statistics: number and percentage for qualitative values. Statistical differences between independent groups were tested using the Chi Square test for qualitative variables while dependent group comparisons were done using Cochrane and MacNemar tests.

3. Results

From a total of 148 *Enterobacteriaceae* isolates [85 (57.4%) *K. pneumoniae*, 42 (28.4%) *E. coli*, and 21 (14.2%) *P. mirabilis*], that were obtained from 148 clinical samples from ICU

admitted patients with suspected nosocomial infections [55 (37.2%) from sputum, 39 (26.3%) from pus aspirates, 29 (19.6%) from urine, and 25 (16.9%) from blood].

All isolates were screened for cefoxitin resistance strains using cefoxitin disk 30-ug. AmpC positive strains were selected for confirmation of AmpC β-lactamase phenotypically by AmpC E test (AB bio-Mérieux, Sweden), the disk approximation test, and Amp C EDTA disc.

From the 148 total isolates, 50 (33.8%) were AmpC β-lactamase isolates [23/50 (46%) *K. pneumoniae*, 16/50 (32%) *E. coli*, 11/50 (22%) *P. mirabilis*], that were obtained from clinical isolates as follows: 20/55 (36.4%) from sputum, 12/39 (30.8%) from pus aspirates, 9/29 (31%) from urine, and 9/25 (36%) from blood (Table 1). For 50/148 (33.8%) AmpC isolates, all phenotypic AmpC tests gave a positive result for AmpC production.

Molecular detection of plasmid mediated AmpC by multiplex PCR was conducted on the 50 AmpC β-lactamase isolates.

Among the 50 AmpC β-lactamase isolates, plasmid encoded AmpC genes were detected by multiplex PCR in 46 (92%) isolates, which included *K. pneumoniae* ($n = 22$) (47.8%), *E. coli* ($n = 15$) (32.6%), and *P. mirabilis* ($n = 9$) (19.6%). One *E. coli,* one *K. pneumoniae*, and two *P. mirabilis* isolates showed no AmpC gene (Table 2).

The most prevalent AmpC gene was that belonging to family CMY-1 which was detected in 73.9% (34/46).

Genotyping of PCR products from amplification of plasmid AmpC genes showed that the CMY genes from 15 *K. pneumoniae* isolates, 12 *E. coli* isolates, and 7 *P. mirabilis* isolates were homologs to CMY-1 gene. CMY-2 genes were detected in 12 *K. pneumoniae*, 9 *E. coli*, and 5 *P. mirabilis* isolates.

Genotyping for PCR product showed that FOX-1 genes were detected in 9 *K. pneumoniae*, 5 *E. coli*, and were not detected in *P. mirabilis* isolates. No genes belonging to the ACC-1 were detected in all isolates. Gene belonging to CMY family was the only one found in *P. mirabilis*.

There were 74 AmpC genes detected in the 92% (46/50) of the positive phenotypic screened isolates. MOX group genes (including CYM-1) were the predominant type in all isolates ($n = 34/46$, 73.9%) followed by CIT group genes (including CMY-2) ($n = 26/46$, 56.5%) then FOX group genes (include FOX-1) ($n = 14/46$, 30.4%).

CMY genes were predominantly present in *E. coli* (CMY-1, $n = 12/15$, 80%) (CMY-2, $n = 9/15$, 60%) and *P. mirabilis* (CMY-1, $n = 7/9$, 77.8%) (CMY-2, $n = 5/9$, 55.6%), but were also found in *Klebsiella* spp. (CMY-1, $n = 15/22$, 68.2%) (CMY-12, $n = 12/22$, 54.5%). FOX-1 genes were present predominantly in the *Klebsiella* spp. ($n = 9/22$, 40.9%) but also found in *E. coli* isolates ($n = 5/15$, 33.3%), and not found in *P. mirabilis*.

In some of these isolates there were more than one gene detected. As regards *K. pneumoniae*, five isolates have both CMY-1 and CMY-2, 7 isolates have both CMY-1 and FOX-1 and 2 isolates have the three genes CMY-1, CMY-2 and FOX-1. For *E. coli* 6 isolates have both CMY-1 and CMY-2, 3 isolates have CMY-1, FOX-1 and 2 isolates have the three genes CMY-1, CMY-2 and FOX-1. For *P. mirabilis* 3 isolates have both CMY-1 and CMY-2 (see Fig. 1).

Table 1 AmpC β-lactamase *Enterobacteriaceae* isolates.

Isolates	Total no.	AmpC positive isolates	%
Klebsiella pneumonia	85	23	27.1
E. coli	42	16	38.1
Proteus mirabilis	21	11	52.4
	148	50	33.8

4. Discussion

Drug resistance poses a therapeutic problem not only in the hospital settings, but also in the community as most of the bacteria have acquired resistance to multiple antibiotics. The various mechanisms of drug resistance in Gram negative bacteria include extended spectrum β-lactamases (ESBL) production, AmpC β-lactamase production, efflux mechanism and porin deficiency. In the clinical laboratory settings, the commonly detected enzymes causing resistance are AmpC β-lactamases and ESBLs. Clinical relevance of AmpC β-lactamases lies in the fact that they confer resistance to both narrow and broad spectrum cephalosporins, β-lactam/β-lactamase inhibitor combinations and aztreonam [10].

Detection of AmpC is important to improve the clinical management of patients suffering from infections and would also provide us with sound epidemiological data. However, there are no clinical and laboratory standard institute guidelines for detection of AmpC mediated resistance in gram negative clinical isolates and hence, it usually poses a problem due to misleading results, especially so in phenotypic tests [13].

In this study, the prevalence of Amp-C producing strains with multiplex PCR from the 50 isolates that were detected as Amp-C positive by phenotypic methods were 92% (46/50) from which 44% (22/50) in the *Klebsiella* spp., 30% (15/50) in *E.* coli and 18% (9/50) in *P. mirabilis*. This rate of prevalence was much higher than the result of Wassef et al. [26], who reported that 26.9% harbored the plasmid mediated AmpC gene and that of Yilmaz et al. [29], who reported that AmpC-producing strains were 10.9% (21/191) in *E. coli* and

Table 2 Distribution of *ampC* genes (CMY-1 and 2, FOX-1 and ACC-1) within study isolates.

Organism	No. (%) of isolates					
	AmpC genes positive	CMY positive		FOX-1 positive	ACC-1 positive	*AmpC* genes negative
		CMY-1	CMY-2			
Klebsiella pneumoniae	22 (47.8%)	15 (68.2%)	12 (54.5%)	9 (40.9%)	0 (0)	1 (4.5%)
E. coli	15 (32.6%)	12 (80%)	9 (60%)	5 (33.3%)	0 (0)	1 (6.7%)
Proteus mirabilis	9 (19.6%)	7 (77.8%)	5 (55.6%)	0 (0)	0 (0)	2 (22.2%)

have both CMY-1 and CMY-2.

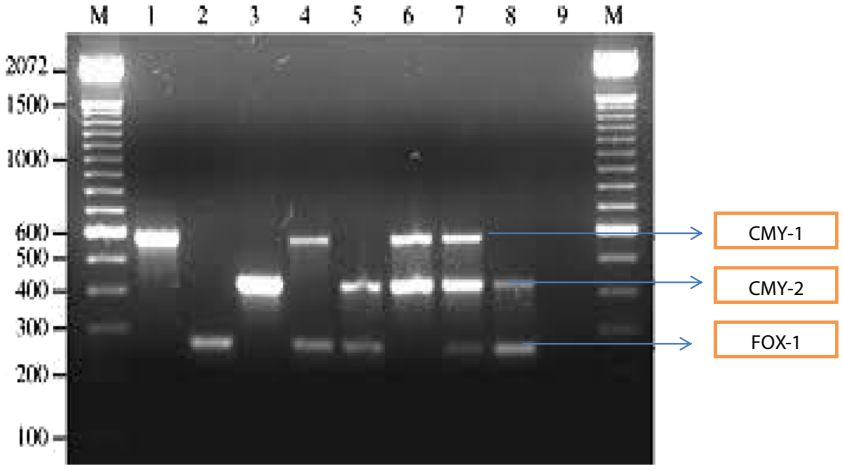

Figure 1 Agarose gel electrophoresis of AmpC β-lactamase genes amplified from nine different isolates. Lane M is 100 bp DNA markers. Lanes 1, 2 and 3 show amplified product of one Amp-C gene. Lanes 4, 5, 6 and 8 gene show amplified products of two Amp-C genes. Lane 7 shows amplified product of one Amp-C gene. While in lane 9 there is no amplified product for any of Amp-C genes.

3.6% (3/82) *K. pneumoniae* in the tested population by PCR. The great difference in the results of these two studies was because in this study the prevalence was calculated from the 50 isolates that were detected as Amp-C positive by phenotypic methods while in the study of Yilmaz et al. [29], it was calculated from whole isolates. Further population-based prevalence studies are required to observe the true spread of AmpC β-lactamases.

In this study, there were 74 Amp-C genes detected in the 92% of the positive phenotypic screened isolates. MOX group genes (including CYM-1) were the predominant type in all isolates (73.9%) followed by CIT group genes (including CMY-2) (56.5%) then FOX group genes (include FOX-1) (30.4%) compared to the result of Fam et al. [11], who reported that, in clinical isolates of *Enterobacteriaceae* from Cairo, Egypt Amp-C genes were detected in 28.3% of the study population including *E. coli, Klebsiella* and *P. mirabilis*. CMY-2 enzyme was found disseminating in all 6 AmpC-positive *E. coli* and in 6/10 of *Klebsiella* species. Only one *K. pneumoniae* isolate harbored CMY-4 while DHA-1 was detected in 3 *Klebsiella* and in one *P. mirabilis* isolate. On the other hand the result of Montgomery et al. [19], who reported that 22 AmpC genes were detected in 25.8% of the positive cefoxitin screened isolates of which 40.9% belonged to each of the MOX and the FOX families, 13.6% belonged to the EBC family, and 4.5% belonged to the CIT family.

In this study, both CMY-1 and CMY-2 were the most common genes detected in our region while the study of Fam et al. [11], reported that CMY-2 and DHA-1 were the most common gene clusters of AmpC in our region while other studies reported that DHA-type enzymes have been previously identified in Taiwan [24]and in China [28]. In Korea DHA-, CMY/MOX-, and ACT-1/MIR-1-type enzymes have also been identified [16].While in the United States, in addition to the types mentioned above, DHA-, ACT-1/MIR-1, and FOX-type enzymes have been identified [23]. In Japan, MOX-1, CMY-9, CMY-19, CFE-1, CMY-2 and DHA-1 have been found in clinical isolates [18]. Enzyme type CMY-2 is widely distributed geographically. It has been reported in Algeria, France, Germany, Greece, India, Pakistan, Taiwan, Turkey, United Kingdom and the United States. Several studies for the detection of AmpC β-lactamase producers in many countries (Saudi Arabia, Taiwan, Korea, North and South America) revealed geographical discrepancy in AmpC β-lactamase types [27].

In our study, CMY genes were predominantly present in *E. coli* (CMY-1, 80%) (CMY-2, 60%) and *P. mirabilis* (CMY-1, 77.8%) (CMY-2, 55.6%), but were also found in the *Klebsiella* spp. (CMY-1, 68.2%) (CMY-12, 54.5%). FOX-1 genes were present predominantly in *Klebsiella* spp. (40.9%) but also found in *E. coli* isolates (33.3%), and not found in *P. mirabilis* however there were detected in another study by Al-Agamy [2]who detected CMY-2 in one *K. pneumoniae* isolate while other genes were not detected. In another study, CMY-2 has been described in *Salmonella enterica* serovar Typhimurium [1]. However, Ktari et al. [15], reported detection of CMY-4 AmpC β-lactamase in *K. pneumoniae* isolate in a Tunisian university hospital.

In our study, in some of these isolates, there is more than one gene. As regards *K. pneumoniae*, five isolates have both CMY-1 and CMY-2, 7 isolates have both CMY-1 and FOX-1 and 2 isolates have the three genes CMY-1, CMY-2 and FOX-1. For *E. coli* 6 isolates have both CMY-1 and CMY-2, 3 isolates have CMY-1, FOX-1 and 2 isolates have the three genes CMY-1, CMY-2 and FOX-1. For *P. mirabilis* 3 isolates have both CMY-1 and CMY-2 Wassef et al. [26], also reported one isolate (*Klebsiella*) harbored 3 genes (*bla*FOX, *bla*MOX and *bla*CIT).

5. Conclusions

The gold standard for the detection of AmpC activity was taken to be a combination of the phenotypic and genotypic testing results. Since clinical laboratories are first to encounter bacteria with new forms of antibiotic resistance, they need appropriate tools to recognize these bacteria, including trained staff with sufficient time and equipment to follow up important observations. Because bacterial pathogens are constantly changing, training must be an ongoing process. The methods

and training that were previously adequate may no longer be sufficient against the newer types of pathogens.

Identification of types of AmpC may aid in hospital infection control and help the physician to prescribe the most appropriate antibiotic, thus decreasing the selective pressure, which generates antibiotic resistance. Sequencing and typing the strains may be required to better understand the genetic relatedness and the molecular epidemiology of this resistance mechanism.

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