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Prevalence of Plasmid-Mediated *ampC* Genes in Clinical Isolates of Enterobacteriaceae from Cairo, Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the prevalence of acquired pAmpCs in clinically important and relevant enterobacterial species and to characterize the molecular types of pAmpC present in our geographic area.
Methodology: Sixty Enterobacterial clinical isolates resistant to third generation cephalosporins and to cephamycins were included in the study. Samples were collected for a period of 6 months between July 2008 and December 2008 from Theodor Bilharz Research Institute (TBRI), Egypt. Bacterial species were identified using API E20. AmpC genes clusters: (bla ACC, bla EBC, bla FOX, bla CMY, bla MOX, and bla DHA) were tested by PCR and DNA sequencing. Clonal relatedness of AmpC-producing *Klebsiellae* isolates was determined by Pulsed Field Gel Electrophoresis (PFGE).
Results: AmpC genes were detected in 28.3% (17/60) of the study population including *E. coli, Klebsiella* and *Proteus mirabilis (P mirabilis).* CMY-2 enzyme was found disseminating in all 6 AmpC-positive *Escherichia coli (E. coli)* and in 6/10 of *Klebsiellae* species. Only one *Klebsiella pneumonia (K. pneumonia)* isolate harbored CMY-4 while DHA-1 was detected in

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3 *Klebsiellae* and in one *P. mirabilis* isolate. PFGE patterns showed no clonal relatedness among the 6 CMY-2-positive *Klebsiella* isolates.
Conclusion: Plasmid-mediated AmpC enzymes are important mechanisms of resistance to ß- lactam drugs. CMY-2 and DHA-1 are the most common gene clusters of pAmpC in our region. AmpC-type resistance in our hospital setting is not due to the dissemination of clonal strains but due to the spread of resistant genes. This is the first report from Egypt identifying DHA-1 and CMY-4 in enterobacterial isolates.

Keywords: AmpC β-lactamases; Klebsiella spp.; E. coli; P. mirabilis; PCR; DNA sequencing; PFGE.

1. INTRODUCTION

Plasmid-mediated ampC β-lactamases (PMABLs), originating from chromosomally-located *ampC* genes of different Gram-negative bacteria, have emerged since the 1980s. PMABL production is one of the mechanisms of resistance to β-lactam antibiotics in Gram negative bacteria conferring resistance to a wide variety of β-lactam antibiotics including 7-α-methoxy cephalosporins (cefoxitin or cefotetan), oxyimino cephalosporins (cefotaxime, ceftazidime, ceftriaxone), monobactam (aztreonam) and are not inhibited by clavulanic acid [1,2,3]. Horizontal gene transfer is widely recognized as the mechanism responsible for the widespread distribution of antibiotic resistance genes, gene clusters encoding biodegradative pathways and pathogenicity determinants in bacteria, especially in Gram-negative pathogens [4]. Most plasmid-mediated AmpC genes are expressed constitutively even in the presence of a complete system for induction and the transfer of chromosomal genes to plasmids allowed the expression of AmpC β-lactamases in *Klebsiella* spp., *E. coli*. *P. mirabilis* and *Salmonella* spp. [5]. Resistance due to pampC enzymes is less common than Extended spectrum β-lactamases (ESBLs) production in most parts of the world, however they may be both harder to detect and broader in their spectrum of resistance activity and they are of special concern because self-transmissibility permits their spread among different bacteria [6,7].

PMABLs can be divided into five structurally distinct clusters; the *Citrobacter freundii* cluster, represented by CMY-2, the *Enterobacter* spp*.* cluster with MIR-1 and ACT-1, the *Morganella morganii* group with DHA-1, the *Hafnia alvei* cluster represented by ACC-1, the *Aeromonas* spp. cluster with MOX-1 (also called CMY-1) and FOX-1 enzymes which constitute two distinct subgroups [8]. The bla_{CMY-2} gene is the most prevalent and has been reported worldwide. Interestingly, bla_{CMY-2} is found in many different plasmid backgrounds, suggesting that it can be mobilized as a part of a smaller transferable fragment [3,9].

Testing PMABL is not routinely done by many laboratories; phenotypic tests may be ambiguous and unreliable resulting in misreporting and treatment failures. In addition, the co-existence of ESBLs may mask phenotypic detection. In addition, there are no Clinical Laboratory Standards Institute (CLSI) guidelines available for optimal detection and confirmation of PMABL. Phenotypic tests do not differentiate between chromosomal and p*amp*C genes. Hence, genotypic characterization is considered the gold standard [8].

The aim of this study was to determine the prevalence of PMABLs in clinically important enterobacterial species and to characterize the specific genes encoding the different pAmpC enzymes.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates and Antimicrobial Susceptibility

Sixty enterobacterial isolates causing infections were prospectively collected for a period of six months, from July 2008 to December 2008, from hospitalized patients or patients attending the outpatient clinic of Microbiology Department of Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. Bacterial species identification was confirmed by using the analytical profile index for *Enterobacteriaceae* (API-20E) (Bio-Mérieux SA, France). The isolates were selected according to the inclusion criteria including species that are known to lack chromosomal ampC (*Klebsiella* spp. and *P. mirabilis*) or are known to minimally express a chromosomal AmpC enzyme (*E. coli*), isolates showing decreased susceptibility to cephamycins indicated by cefoxitin (FOX) intermediate (15-17 mm) or resistance phenotype (≤14 mm) [10] and resistance to third generation cephalosporins (e.g. ceftazidime) with or without inhibition by clavulanic acid. Isolates were screened by the Kirby-Bauer (KB) disk diffusion method according to the criteria published by the CLSI [10]. Inhibition by clavulanic acid was detected simultaneously with antimicrobial susceptibility analysis using modified double disk synergy test [11]. The following antibiotics were tested: cefoxitin (FOX; 30µg), cefotaxime (CTX; 30µg), ceftazidime (CAZ; 30µg), cefepime (FEP; 30µg), aztreonam (ATM; 30µg), amoxicillin-clavulanic acid (AMC; 30/10µg), piperacillin/tazobactam (TZP; 100/10µg), ampicillin (AMP; 10 µg) and cephalothin (CL; 30 µg) (Bio-Rad, France). Suspensions of test isolates were prepared by inoculating the organism in 5ml sterile saline. Saline turbidity was adjusted to the equivalent of 0.5 McFarland turbidity standards (Bio-Mérieux, France). Bacterial suspensions were plated on Mueller-Hinton agar (MHA). Antibiotic disks of FOX, CAZ, CTX, ATM, CL, AMP, TZP and FEP were arranged in proximity (30mm centre to centre) with the AMC disk using an 8-cartridge antibiotic disc dispenser (Bio-Rad, France). The plates were incubated for 18-24 h at 37˚C. Results of antimicrobial susceptibility were interpreted according to CLSI guidelines: FOX (S≥18), CL (S≥18), CTX (S≥23), CAZ (S≥18), FEP (S≥18), ATM (S≥22), AMC (S≥22), TZP (S≥21) and AMP (S≥17) [10]. Test organisms showing synergy between the AMC disk and any cephalosporin disk or the ATM disk and/or between TZP and FEP was diagnosed as ESBL-producing strains. Synergy detected in the form of “bouchon-champagne”, “key-hole appearance” or "lens appearance" between the expected disks was interpreted as positive for ESBL production. The standard strains *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively, for the assay.

2.2 Detection of bla ampC by PCR

Bacterial DNA was extracted using DNA QIAmp DNA mini kits (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. Genes encoding PMABLs were amplified using previously described primers [Table 1] except for the MOX primers; these were designed specifically for this study using representative sequences from Genebank [12]. Melting temperature (Tm) of MOX primers used was 55 ˚C for 30sec. Nucleotide sequences for pAmpC genes were retrieved from GenBank database using basic local alignments software (BLAST) algorithm available at the National Center of Biotechnology (NCBI) Information Web site *(http://www.ncbi.nlm.nih.gov)*. Sequences were assembled using BioEdit (version 7.0.5.3) available at *http://bioedit.software.informer.com* and phylogenitic analysis dendrogram were designed using the Clustal X application within the BioEdit. The established dendogram harbored the 6 major clusters of pAmpC genes. Accession numbers; CMY-10_Kp_AF381618, CMY-11_Ec_AF381626, MOX-1_Kp_D13304, CMY-

8_Kp_AF167990, CMY-9_Ec_AB061794, MOX-2_Kp_AJ276453, CMY-1_Kp_X92508, CMY-10_Kp_AF381618, CMY-11_Ec_AF381626, MOX-1_Kp_D13304, CMY-8_Kp_AF167990, CMY-9_Ec_AB061794.

2.3 DNA Sequencing

PCR *ampC*-positive amplicons were purified using QIA quick PCR purification kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Sequencing reactions were carried out using the Perkin Elmer Big Dye kit. v3.1 (Applied Biosystems; Foster City, CA, USA). DNA sequences were assessed using software included in the Bioedit software package and phylogenetic analyses were performed using representative sequences retrieved from Genbank using the software program Mega4.0 [15].

2.4 Molecular Relatedness of ampC-Producing Strains

Clonal relatedness of selected AmpC producing *Klebsiella* isolates was analysed by pulsed field gel electrophoresis, PFGE following a standard protocol [16,17]. Briefly, an agarose plug containing intact genomic DNA representing a single *Klebsiella* isolate was digested with XbaI (New England BioLabs, USA). Genomic DNA was separated using multidirectional gel electrophoresis supplied by a Chef-mapper electrophoresis cell (Bio-Rad Laboratories, USA). Electrophoresis was conducted at a constant temperature of 14℃ with a pulse time ramped linearly from 5-35 sec at 6 V/cm in 0.5x TBE buffer for 22 h. Clonal relatedness was interpreted by the criteria of Tenover et al. [18].

2.5 Statistical Methods

Data were statistically described in terms of frequencies (number of cases) and relative frequencies (percentages). A probability value (P value) less than 0.05 was considered statistically significant. All statistical calculations were done using Microsoft Excel 2007 (Microsoft Corporation, New York, USA), SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) or Quick Calcs Online calculators for scientists (Graph pad software Inc., San Diego, CA, USA).

Table 1. Primers for different plasmid-mediated AmpC β-lactamase clusters

Primer name	Targets	Sequence (5'-3')	Amplicon size (bp)	Reference
ACC-F	ACC	AAC AGC CTC AGC AGC CGG TTA	346	[13]
ACC-R	ACC	TTC GCC GCA ATC ATC CCT AGC	346	[13]
EBC-F	MIR-1T and ACT-1	TCG GTA AAG CCG ATG TTG CGG	302	[13]
EBC-R	MIR-1T and ACT-1	CTT CCA CTG CGG CTG CCA GTT	302	[13]
FOX-F	FOX-1 to FOX-5b	AAC ATG GGG TAT CAG GGA GAT G	190	[13]
FOX-R	FOX-1 to FOX-5b	CAA AGC GCG TAA CCG GAT TGG	190	[13]
CMY-F	LAT-1toLAT-4,CMY-2 to CMY-7 and BIL-1	TGG CCG TTG CCG TTA TCT AC	870	[14]
CMY-R	LAT-1to LAT-4,CMY-2 to CMY-7 and BIL-1	CCC GTT TTA TGC ACC CAT GA	870	[14]
MOX-F	MOX-1and MOX-2, CMY-1 and CMY-8 to CMY-11	GCT CAA GGA GCA CAG GAT	1000	designed for this study
MOX-R2	MOX-1and MOX-2, CMY-1 and CMY-8 to CMY-11	CAG GAT GGC GTG GGC CGC	1000	designed for this study
DHA-F	DHA-1 and DHA -2	GCC GGT CAC TGA AAA TAC AC	762	[14]
DHA-R	DHA-1 and DHA-2	TAC GGC TGA ACC TGG TTG TC	762	[14]

3. RESULTS

3.1 Bacterial Isolates and Clinical Specimens

Sixty clinical Gram-negative bacteria resistant to cephamycins and to third generation cephalosporins with or without inhibition by clavulanic acid were tested for PMABLs. The most frequently isolated species were *E. coli* followed by *Klebsiella* then *P. mirabilis* (34/60; 57%; 23/60; 38% and 3/60; 5% respectively). The isolates were recovered from urine specimens (38/60; 63%), pus (10/60; 17%), sputum (7/60; 12%) and body fluids (5/60; 8%). Specimens collected from patients admitted to nephrology, urology, surgery and gastroenterology wards represented 61.7% (37/60) of total samples, while 21.7% were from patients admitted to ICU (13/60) and 16.6% from patients attending outpatient clinic (10/60).

In AmpC-positive isolates, the resistance to aminopenicillins and to third generation cephalosporins was high, reaching 100% for ampicillin, cefotaxime and ceftazidime and were resistant to fourth generation cephalosporins (cefepime), aztreonam, amoxicillin-clavulanic acid and piperacillin/tazobactam in 58.8%, 88.2%,100% and 41.1% respectively. In the AmpC-negative isolates, resistance to cephalosporins was also high: 95.3% to ampicillin, 86% to cefotaxime, 90.6% to ceftazidime and 83.7% to cefepime. They were resistance to amoxicillin-clavulanic acid, aztreonam and piperacillin/tazobactam in 97.6%, 86% and 53.5% respectively [Table 2].

3.2 Detection of bla ampC Genes by PCR

Seventeen (28.3%) out of the 60 collected isolates were *bla*$_{ampC}$ positive by PCR. The most commonly detected *ampC* type were *bla*$_{CMY}$ (13/17; 76.5%). Amplicons with predicted size of 870 bp were generated. The remaining four (23.5%) *bla*$_{ampC}$ positive isolates belonged to the DHA family with predicted amplicon size of 762 bp. No *bla*$_{ampC}$ genes belonging to the *bla*$_{ACC}$, *bla*$_{FOX}$, *bla*$_{MOX}$ or *bla*$_{EBC}$ families were detected (data not shown). AmpC-producing *Klebsiella* spp. were significantly more prevalent than *E. coli* (43.5% versus 17.6%) (p=0.03; [Table 3].

3.3 Sequence Analysis and Characterization of ampC Genes

AmpC nucleotide sequences retrieved from the 17 AmpC-positive isolates were analyzed to determine their relationship to other *bla*$_{ampC}$ genes available in GenBank database using the BLAST nucleotide algorithm *(*http://www.ncbi.nlm.nih.gov) sequences of the 12/13 *bla*$_{CMY}$-positive isolates were indistinguishable from an *E. coli* pAmpC β-lactamase *bla*$_{CMY2}$ gene (GenBank accession no. EF406116.1); the remaining isolate was indistinguishable to the *E. coli* plasmid-encoded cephamycinase (*bla*$_{CMY-4}$) gene (accession no. AF420597.1). Sequences of the four *bla*$_{DHA}$-positive isolates were indistinguishable to the *K. pneumoniae* plasmid β-lactamase *bla*$_{DHA-1}$ (GenBank accession no. AY585202.1) (Fig. 1). Most *bla*$_{ampC}$ -positive isolates (10/17) were found in *Klebsiella* spp. Six out of the ten *bla*$_{ampC}$ -positive *Klebsiella* isolates were of *bla*$_{CMY2}$ type (four of them were in *K. pneumoniae* and two in *K. oxytoca*.); three were of *bla*$_{DHA-1}$ type (all were isolated from *K .pneumoniae* spp.) which represents 75% (3/4) of all identified *bla*$_{DHA}$ -positive isolates. Only one *K. pneumoniae* isolate harbored *bla*$_{CMY-4}$. The *bla*$_{ampC}$ -positive *P. mirabilis* isolate harbored a *bla*$_{DHA-1}$ type *bla*$_{ampC}$ gene. Interestingly, all six *bla*$_{ampC}$ -positive *E. coli* isolates were of *bla*$_{CMY2}$ type [Table 3].

Table 2. Antimicrobial resistance of 60 studied Enterobacteriaceae clinical isolates according to AmpC-production

Strain (no.)	AMP	CTX	CAZ	FEP	ATM	AMC	TZP
	No. %	No. %	No. %	No. %	No. %	No. %	No. %
***E.coli* (no=34)**							
AmpC +ve **(no.** 6)	6(100)	6(100)	6(100)	2(33.3)	5(83.3)	6(100)	1(16.7)
AmpC –ve (**no.**28)	26(92.9)	24(85.7)	26(92.9)	24(85.7)	25(89.2)	27(96.4%)	12(42.9)
***Klebsiella* spp. (no.=23)**							
AmpC +ve (**no.**10)	10(100)	10(100)	10(100)	8(80)	10(100)	10(100)	6(60)
AmpC –ve (**no.**13)	13(100)	13(100)	12(92.3)	12(92.3)	12(92.3)	13(100)	11(84.6)
***P.mirabilis* (n=3)**							
AmpC +ve (**no.**1)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)
AmpC –ve (**no.**2)	2(100)	0(0)	1(50)	0(0)	0(0)	2(100)	0(0)
Total (no.60)	58(96.6)	54(90)	56(93.3)	46(76.6)	52(86.6	59(98.3)	30(50.0)
AmpC +ve (no.=17)	17(100)	17(100)	17(100)	10(58.8)	15(88.2)	17(100)	7(41.1)
AmpC –ve (no.=43)	41(95.3)	37(86)	39(90.6)	36(83.7)	37(86)	42(97.6)	23(53.5)

AMP: ampicillin, CTX: cefotaxime, CAZ: ceftazidime, FEP :cefepime, ATM: aztreonam, AMC amoxicillin-clavulanic acid, TZP : piperacillin/tazobactam.

Table 3. Prevalence and types of ampC genes according to species

Isolate	Frequency	AmpC-positive No. (%)	Type of bla_{ampC}		
			CMY-2	CMY-4	DHA-1
E. coli	**34**	**6 (17.6)**	**6**	**-**	**-**
***Klebsiella* spp.**	**23**	**10 (43.5)**	**6**	**1**	**3**
K.pneumoniae	15	8	4	1	3
K.oxytoca	2	2	2	-	-
K.ornithinolytica	6	-	-	-	-
P.mirabilis	**3**	**1**	**--**	**-**	**1**
Total (%)	**60**	**17(28.3)**	**12(70.5)**	**1(5.8)**	**4 (23.5)**

3.4 Clonal Relatedness among AmpC-Positive *Klebsiella* spp.

The genomic DNA was digested using the restriction enzyme (XbaI) and analyzed by PFGE. XbaI PFGE pattern of the six blaCMY-2-positive *Klebsiella* isolates (4 *K.pneumoniae* and 2 *K.oxytoca)* showed no similarities between the isolates [Fig. 1].

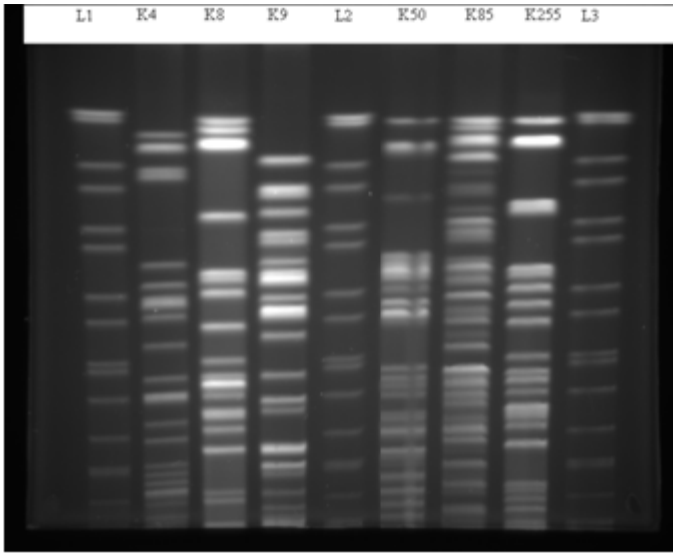

Fig. 1. XbaI PFGE profile of bla_{CMY-2}-positive *Klebsiella* isolates. Lanes 1, 5, 9: S. brander up molecular weight ladder, Lanes 2, 3, 4, 6,7, 8: *Klebsiella CMY-2* positive isolates

4. DISCUSSION

The increasing variety of β-lactamases produced by isolates of the family *Enterobacteriaceae* raises concern about our dependence on β-lactam drugs and the emergence of pan-resistant species [9]. AmpC β-lactamases represent a group of clinically important β-lactamases that are naturally produced by a variety of Gram-negative bacteria [3]. Resistance to expanded-spectrum cephalosporins in *Enterobacter* spp., *Citrobacter* spp., and Serratia spp. may develop through the expression of chromosomally encoded class C beta-lactamases.

Although a chromosomal *ampC* gene is present in *E.coli*, it is not usually expressed because of the presence of a transcriptional attenuator coupled with a weak promoter [19].

Horizontal gene transfer mediated by R plasmids, transposons and integrons is largely responsible for increasing the incidence of antibiotic-resistant infections worldwide [20]. The transfer of *ampC* genes to plasmids has resulted in their dissemination among *Enterobacteriaceae*, with the consequence that *ampC*-encoded beta-lactamases are now present in strains of *Klebsiella* spp., *E. coli*, *P. mirabilis*, and *Salmonella* spp. [19].

In the present study PCR was used to detect the prevalence of bla_{AmpC} genes and to identify mechanisms of resistance in selected species. The prevalence rate was 28.3% and *ampC* genes were significantly more prevalent in *Klebsiella* spp. (43.4%) than in *E. coli* (17.6%).The most prevalent and most widely distributed PMABL enzyme types were the CMY type. DHA-type enzymes have been previously identified in Taiwan [19] and in China [21]. In Korea, DHA-, CMY/MOX-, and ACT- 1/MIR-1-type enzymes have also been identified [22,23] while in the United States, in addition to the types mentioned above, DHA-, ACT-1/MIR-1, and FOX-type enzymes have been identified [24,25]. In Japan, MOX-1, CMY-9, CMY-19, CFE-1, CMY-2 and DHA-1 have been found in clinical isolates [26]. 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 [27].

In the present study , amplification of DNA by PCR using different primers for pAmpC clusters revealed that CMY was the most predominant gene (76.5%) followed by DHA (23.5%). These results were in agreement with two studies from Spain and Singapore in which CMY-2 was found in 66.7% and 79% and DHA-1 in the 25.6% and 21% respectively [29,19].

DNA sequence analysis showed that CMY-2 enzyme was found in all six AmpC-positive *E. coli* and in 6/10 of *Klebsiellae* species, while DHA- 1 in 3 *Klebsiellae* and in one *P. mirabilis* isolate. One *K. pneumoniae* isolate harbored CMY-4. These findings were consistent with previous studies confirming pAmpC β- lactamases of the CMY-2 type in *E. coli* as a major factor contributing to AmpC resistance phenotype [30,31]; whereas DHA-1 was predominantly found in *K. pneumoniae* isolates [19,27,29,32]. Similar findings were reported from Canada, where 13.5 % of their isolates harbored CMY-2 gene [31] and also in Japan where out of 29 PABL-positive strains, 20 (69.0%), 6 (20.7%), 2 (6.9%) and 1 (3.4%) isolates carried the genes for CMY-2, DHA-1, CMY-8, and MOX-1 respectively. Our results showed that three *K. pneumoniae* isolates and one of three *P. mirabilis* isolates carried the *bla* DHA-1 gene. A recent study by Song et al. [33] reported that AmpC producers were detected in 8/222 *P. mirabilis* isolates (3.6%) and none was a DHA-1; while Adler et al [34] found DHA-1 producers at a low rate (0.75%) in *Proteus* species. The emergence of such inducible pAmpC, DHA-1 raises concern because of its association with higher mortality rates compared to other AmpC enzymes [34].

To our knowledge, this is the first report from Egypt identifying DHA-1 and CMY-4 in bacterial isolates. In Egypt, Al-Agamy et al. [35], detected *bla* CMY-2 in one *K. pneumoniae isolate* while other genes were not detected. In another study, PMABL determinants producing AmpC enzymes of CMY-2 have been described in *Salmonella enterica* serovar Typhimurium [36]. However, Ktari et al. reported *detection* of CMY-4 AmpC β-lactamase in *K. pneumonia* isolate in a Tunisian university hospital [37] and Decré et al. detected CMY-4 gene in one of two *K. pneumonia* isolated in four hospitals in Paris area [38]. Molecular typing techniques, particularly macrorestriction patterns of total genomic DNA, obtained by PFGE, are now

widely accepted as an epidemiological tool and have been applied for isolate comparison and characterization in outbreaks [39]. On comparing the clonal relatedness of our AmpC-positive CMY-2 *K. pneumoniae* isolates by PFGE, no clonal relatedness could be detected. This emphasizes that the transferable AmpC-mediated resistance in our hospital setting is the result of transfer of a mobile element rather than the spread of a single clone. A similar observation was noted by Roche et al. [40]. In another study clonal typing showed that isolates producing DHA-1 or CMY-2 were of diverse clonal types although there were some outbreak strains identified among *K. pneumoniae*. Interestingly the investigators in that study observed identical or similar enterobacterial repetitive intergenic consensus sequence (ERIC) patterns among isolates from different hospitals, suggesting that inter-hospital transmission was occurring [32]. Yamasaki et al. [27], noted that the pattern analysis of randomly amplified polymorphic DNA and PFGE analysis revealed that the prevalence of CMY-2-producing *E. coli* strains was not due to epidemic strains and that three DHA-1-producing *K. pneumoniae* strains were identical, suggesting their clonal relatedness. However, PFGE was not done to DHA-positive *Klebsiella* isolated in our study.

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 [6].

5. CONCLUSION

Plasmid-mediated ampC enzymes are important mechanisms of resistance to β- lactam drugs. In our institute, CMY-2 and DHA-1 were the most common gene clusters of pAmpC. To our knowledge, this was the first report from Egypt identifying DHA-1 and CMY-4 in enterobacterial isolates. AmpC-type resistance in our hospital setting could be due to the spread of resistant genes as there was no evidence of dissemination of clonal strains. Therefore, further in vitro experiments are recommended to know if these ampC are transferable.

CONSENT

The study protocol was approved by Theodor Bilharz Research Institute research ethics committees. Bacterial isolates were prospectively collected from microbiology department of Theodor Bilharz Research Institute and ethics committee waived the need for consent. The study was conducted in accordance with the ethical guidelines of the 1975 declaration of helsinki and international conference on harmonization guidelines for good clinical practice

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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