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Prevalence of coexistence genes and clonal spread of ESBL-producing isolates causing hospital- and community-acquired infections in Zenica-Doboj Canton, Bosnia and Herzegovina

Amir Ibrahimagić[1]*, Selma Uzunović[1], Branka Bedenić[2,3]

[1]Department for Laboratory Diagnostics, Institute for Public Health and Food Safety, Zenica, Bosnia and Herzegovina, [2]Department of Medical Microbiology and Parasitology, School of Medicine, University of Zagreb, Zagreb, Croatia, [3]Department for Clinical and Molecular Microbiology, Clinical Hospital Center Zagreb, Zagreb, Croatia

ABSTRACT

Introduction: Coexistence type of extended-spectrum beta-lactamases (ESBL)-producing isolates is serious problem in the public health world.

Materials and Methods: Antibiotic susceptibility was determined by disc diffusion and broth microdilution according to the Clinical and Laboratory Standards Institute guidelines. Double-disk synergy test was performed to screen for ESBLs/pAmpC beta-lactamases. Polymerase chain reaction (PCR) was used to detect bla_{ESBL}/bla_{ampC}/bla_{carb} genes. Genetic relatedness of the strains was determined by pulsed-field gel electrophoresis.

Results: In this study, 88 of the inpatient isolates (n=126; 10.0%) and 62 of the outpatient (n=184; 6.4%) beta-lactamase-producing isolates were taken for the study. They included 50.0/29.0% *Klebsiella pneumoniae*, 12.5/30.6% *Escherichia coli*, 11.4/4.8% *Acinetobacter baumannii*, 8.0/14.5% Klebsiella oxytoca, 8.0/4.8% *Enterobacter cloacae*, 5.7/8.1% *Proteus* spp., and <3.5% of other isolates. Coexistence of more than two types of beta-lactamases was detected in 77.3% of inpatient and 45.2% of outpatient isolates. Among inpatient isolates, *Klebsiella* spp. and *E. coli* were the most frequent isolates which produce more than two types of genes, in ≈65% and ≈12% of cases. Separately, combination of four TEM+SHV+CTX-M+OXA-1 beta-lactamases in inpatient *K. pneumoniae* isolates was detected in 63.6% of cases, respectively. Differences in antimicrobial resistance were higher to cephalosporin agents in *Klebsiella* spp. and *E. coli* at inpatient and outpatient isolates which produce more than two types of beta-lactamases than in isolates which produce one type of beta-lactamases.

Conclusion: This work demonstrates a progressively increasing prevalence of coexistence type of beta-lactamases, especially in inpatient isolates. Continuous monitoring and surveillance and proper infection control and prevention practice will limit the further spread of these isolates.

Key words: Coexistence type of extended-spectrum beta-lactamases; comparison; antimicrobial resistance

*Corresponding author: Amir Ibrahimagić, Institute for Public Health and Food Safety Zenica Fra Ivana Jukića 2, 72000 Zenica, Bosnia and Herzegovina. Tel.: 0038732/443-580. Fax.: 0038732/443-530. E-mail: ibrahimagic.amir@gmail.com

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UNIVERSITY OF SARAJEVO
FACULTY OF HEALTH STUDIES

INTRODUCTION

Most of the genes encoding extended-spectrum beta-lactamases (ESBLs) are generally found on plasmids that conferred resistance to multiple antibiotic classes and are readily transferable between and within bacterial pathogens (1). Family of cefotaxime beta-lactamases (CTX), after SHV-1 and TEM-1 has been reported with increasing frequency in the world (2). These genes are commonly found with other resistance genes, causing a coresistance profile inclusive of other non-beta-lactam antibiotics such as aminoglycosides and tetracycline (3).

In recent years, the production of more than one beta-lactamase has frequently been reported in some countries, such as in the study from India (4,5), China (6), Iran (7), and Germany (8), Egypt (9), USA (10), Malaysia (11), and Bosnia and Herzegovina (B and H) (12). The antimicrobial resistance of Gram-negative bacteria has risen progressively during the past decades, leading to an increasing number of outbreaks due to the existence of multi-resistant bacteria, especially those producing more than one beta-lactamase type (4). *Escherichia coli* and *Klebsiella pneumoniae* are major agents and also the most frequent bacteria that produce different type of ESBLs. Now, it is a serious problem in the world, because the emergence of multiple-ESBLs is the important cause of transferable multidrug resistance in Gram-negative bacteria (13).

In the study from Zenica-Doboj Canton (B and H) (2015), the coexistence of several ESBL types in the same bacterial strain causing inpatient and outpatient urinary tract infections was found with prevalence rates of 44.4%/50% in *E. coli*, 81.8%/30% in *K. pneumoniae*, and 66.7%/22.2% in *Klebsiella oxytoca*, respectively (12).

The aim of the study was to investigate a prevalence, antimicrobial characteristics, and mode of spread of Gram-negative isolates producing more than two different beta-lactamase types causing in- and out-patient infections in Zenica-Doboj Canton, B and H.

MATERIALS AND METHODS

Setting, bacterial isolates, and study design

All consecutive, non-duplicate Gram-negative isolates collected from different specimens and resistant to expanded-spectrum cephalosporins from various hospital departments, including outpatient department, of the Cantonal Hospital Zenica, B and H, in the period December 2009 - May 2010 were included in the study. The Cantonal Hospital Zenica is 849-bed tertiary level hospital admitting about 25,000 patients/year, with 2,40,000 hospital days, and covers a population of 3,31,229 in Zenica-Doboj Canton.

The Institutional Review Board approval from the Ethics Committee of the Cantonal Hospital Zenica was obtained before the initiation of the study.

Detection of ESBLs, plasmid-mediated AmpC β-lactamases, and carbapenemases

A double-disk synergy test using the combination of amoxicillin/clavulanate with cefotaxime, ceftriaxone, ceftazidime, and cefepime was performed to detect the production of ESBLs (14). Production of ESBLs was confirmed according to the Clinical and Laboratory Standards Institute (CLSI) combined disk test.

E. coli, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Morganella morganii*, and *Proteus* spp. strains resistant to expanded-spectrum cephalosporins, cefoxitin, and beta-lactam combinations with inhibitors were screened for the production of AmpC beta-lactamase. Production of AmpC beta-lactamase was tested by combined disk test using 3-amino phenylboronic acid (PBA) (15).

Production of carbapenemases Group A or Group B was confirmed by combined disk test using EDTA and PBA (16).

Susceptibility testing

Susceptibility testing to 12 antimicrobials was performed by a two-fold microdilution technique according to the CLSI standard procedure: Amoxycillin+clavulanic acid (AMC; 20+10 μg), cefazolin (CZ; 30 μg), cefuroxime (CXM; 30 μg), ceftazidime (CAZ; 30 μg), cefotaxime (CTX; 30 μg), ceftriaxone (CRO; 30 μg), cefoxitin (FOX; 30 μg), cefepime (FEP; 30 μg), imipenem (IMP; 10 μg), meropenem (MEM; 10 μg), gentamicin (GM; 10 μg), and ciprofloxacin (CIP; 5 μg) (14). *E. coli* ATCC 25922 (ESBL negative) and *K. pneumoniae* 700603 (ESBL positive) were used as quality control strains.

Polymerase chain reaction (PCR) detection of bla_{CTX-M}, bla_{SHV}, bla_{TEM}, and bla_{KPC} genes

PCR was used to detect alleles encoding ESBL enzymes.

The presence of bla_{TEM}, $bla_{SHV,}$ and bla_{CTX-M} genes was investigated by PCR using primers and conditions as described previously (17).

Primers IS26F (5'-GCG-GTA-AAT-CGT-GGA-GTG-AT-3) and IS26R (5'-ATT-CGG-CAA-GTT-TTT-GCT-GT-3') were used to amplify 400 bp fragment spanning the link between IS26 insertion sequence and bla_{CTX-M} gene in CTX-M-producing isolate (18). Genes encoding carbapenemases of Group A (KPC), Group B (MBLs belonging to VIM, IMP, and NDM family), and OXA-48 were detected by PCR as described previously (16).

Molecular detection of plasmid-mediated AmpC β-lactamases

Multiplex PCR with primers specific for MOX, CMY, DHA, ACT, MIR, and FOX βlactamases was used to detect plasmid-mediated Amp β-lactamases in strains resistant to cefoxitin and β-lactam/inhibitor combinations (15).

Typing by pulsed-field gel electrophoresis (PFGE) of bacterial DNA

Isolation of genomic DNA, digestion with the *Xba*I restriction enzyme (Invitrogen), and PFGE of the resulting fragments were performed as described by Kaufman (19). The electrophoresis was carried out with a CHEF-DRII apparatus (Bio-Rad Laboratories, Hercules, CA). The PFGE patterns were compared following the criteria of Tenover et al. (20) and analyzed by the GelComparII software (Applied Maths, St Martens, Belgium).

RESULTS

ESBL prevalence

During the period December 2009–May 2010, a total number of 1254 consecutive, non-duplicate inpatient, and 2857 outpatient Gram-negative bacteria were isolated. Among inpatient samples, 126 (10.0%) were beta-lactamase-producing isolates, 40 (31.7%) were from urine samples, 35 (27.8%) from surgical wounds, 15 (11.9%) from skin and soft tissue infections (SSTIs), 14 (11.1%) from cannula and stoma, 13 (10.3%) from upper respiratory tract, 5 (4.0%) from burns, and 4 (3%) from other samples (catheters and ear swabs).

Among outpatient samples, 184 (6.4%) were beta-lactamase-producing isolates, of which 148 (80.4%) were from urines, 30 (16.3%) from surgical wounds, and 6 (3.3%) from other samples (SSTIs, upper respiratory tract, ear swabs, genital tract, and eyes).

A total of 88 inpatient and 62 outpatient isolates were available for further analysis including 44/18 (50.0/29.0%) *K. pneumoniae*, 11/19 (12.5/30.6%) *E. coli*, 10/3 (11.4/4.8%) *Acinetobacter baumannii*, 7/9 (8.0/14.5%) *K. oxytoca*, 7/3 (8.0/4.8%) *E. cloaceae*, 5/5 (5.7/8.1%) *Proteus* spp., and 4/5 (3.5%) of other in/outpatient isolates (*C. freundii*, *M. morganii*, and *P. aeruginosa*).

Most of the 88 ESBL-producing inpatient strains originated from the pediatric and neurology departments, 29 (33.0%) and 18 (20.5%) isolates.

The most common beta-lactamase-producing inpatient isolates were *K. pneumoniae* 44 (50.0%), and *E. coli* 11 (12.5%), followed by ten *A. baumannii*, seven *K. oxytoca*, seven *Enterobacter cloacae*, five *Proteus* spp., three *C. freundii*, and one *P. aeruginosa* (11.4%, 8.0%, 8.0%, 5.7%, 3.4%, and 1.1%, respectively). The most common beta-lactamase-producing outpatient isolates were *E. coli* 19 (30.6%) and *K. pneumoniae* 18 (29.0%), followed by nine *K. oxytoca*, five *Proteus* spp., three *E. cloacae*, three *A. baumannii*, two *C. freundii*, two *M. morgannii*, and one *P. aeruginosa* (14.5%, 8.1%, 4.8%, 4.8%, 3.2%, 3.2%, and 1.6%, respectively).

Thirty-six (of 88, 40.9%) isolates were obtained from the inpatients older than 60 years of age, followed by the group below 1 year of age, 25 (28.4%). Duration of hospitalization (median) was 14 days; in seven cases, duration of hospitalization was in the range 33–54 days. Amoxicillin-clavulanic, gentamicin, and cefazolin were mostly used antimicrobials in the inpatient infections caused by beta-lactamase-producing isolates, in 39, 23, and 22 (44.4%, 26.4%, and 25.0%) cases, respectively.

Twenty-seven (of 62, 43.5%) beta-lactamase-producing isolates were obtained from the outpatients older than 60 years of age, followed by children

up to 1 year of age, 16 (25.8%). Other data for outpatients were missing.

Detection and characterization of β-lactamases

Among the 88 and 62 beta-lactamase-producing in- and out-patient isolates, 61 (69.3%) and 19 (30.6%), respectively, were positive for bla_{CTX-M} gene by PCR.

Sixty-eight (77.3%) inpatient and 29 (46.8%) outpatient isolates coproduced more than two genes in different combinations: bla_{CTX-M}, bla_{TEM-1}, bla_{SHV-1}, bla_{OXA-1}, bla_{DHA-1}, or bla_{CMY-2}.

Forty-five (of 51, 88.2%) inpatient beta-lactamase-producing *Klebsiella* spp. (38 *K. pneumoniae* and 7 *K. oxytoca*) coproduced more than two types of different genes including bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{OXA-1}, bla_{CMY-2}, or bla_{DHA-1} (Tables 1 and 2).

Ten (of 19, 52.6%) outpatient beta-lactamase-producing *E. coli* isolates coproduced more than two types of beta-lactamases (Tables 1 and 2).

Characterization of carbapenemases

Carbapenemases were not detected neither by phenotypic nor molecular tests.

Antibiotic susceptibility

Isolates with one type of beta-lactamases

Prevalence of resistance to all cephalosporin antibiotics was noticed among six inpatient *Klebsiella* spp., six *A. baumanni,* and seven other isolates (one *Citrobacter freundii*, two *E. coli*, three *E. cloacae,* and one *Pseudomonas* spp.) ranging from 14.3% to 100%. Gentamicin and ciprofloxacin also showed the low activity, 42.9–83.3%, respectively. Resistance to imipenem and meropenem in *A. baumanni* was noticed in 83.3% and 16.7%.

Nine *E. coli* and 12 *Klebsiella* spp. outpatient isolates were resistant to cephalosporins, aminoglycosides, and fluoroquinolones, with the resistance rates ranging from 25.0% to 100.0%.

One *A. baumannii* isolate was resistant to imipenem and meropenem (Table 3).

Isolates with more than two types of beta-lactamases

ESBL-producing *E. coli* and *Klebsiella* spp. inpatient isolates which produced two and more than two types of beta-lactamases had higher resistance rates to ceftazidime, cefotaxime, and ceftriaxone with the range of 50–90%, than in isolates which produced one or two types of beta-lactamases. The same isolates were resistant to gentamicin and ciprofloxacin (Table 3).

In outpatient, ESBL-producing *E. coli* and *Klebsiella* spp. isolates, coproducing more than two types of beta-lactamases, had higher resistance rates to ceftazidime and ceftriaxone than isolates with one type of beta-lactamases (Table 3).

PFGE typing

The genetic relatedness was examined through PFGE typing. Forty-one isolates of *K. pneumoniae*, 18 of *E. coli*, 11 of *Proteus* spp., 10 of *E. cloacae*, and six of *C. freundii* isolates were available for the analysis (Figure 1).

DISCUSSION

In the present study, a high prevalence of ESBL-producing isolates which produced two and more than two types of beta-lactamases and their antimicrobial resistance patterns have been reported for the first time in the Zenica-Doboj Canton, B and H.

CTX-M was the most prevalent gene in inpatient ESBL-producing isolates in this study (70%), as it was also reported in some other countries where the prevalence was 96.9% in China (5), 80.3% and 91.3% in Germany (8,21), and 75.5% in Madagascar (22). In outpatients, TEM was the most prevalent gene (50%).

The presence of more than two different types of beta-lactamases in the same isolates in this study was noticed, 77% in inpatient, and 47% in outpatient. This is similar with the report from India, Germany, Malaysia, and Iran where the prevalence of coproduction of more than two types of beta-lactamases in inpatient was 70%, 77%, 50%, and 45% (5,8,11,13), respectively. These prevalences are lower in the report from China (11%), North Iran (30%), and Egypt (10%) (6,7,9).

Coproduction of more than two types of beta-lactamases was most frequently noticed in *Klebsiella* spp. (66%), which is in concordance with the report from India (70%) (5). This result is not in agreement

TABLE 1. Detection of bla_{ESBL} and bla_{AmpC} of the inpatient/outpatient isolates

Causative agent isolated	Total number of the inpatient-outpatient isolates	n (%) of the inpatient/outpatient positive isolates							Number of CTX-M, SHV and AmpC types of the inpatient/ outpatient isolates	Number of isolates co-produced more than 2 genes
		bla_{TEM-1}	bla_{SHV}	bla_{CTX-M}	bla_{OXA-1}	bla_{AmpC}	bla_{OXA-51}	$bla_{KPC/VIM}$		
E. coli	11/19	5 (45.5)/11 (57.9)	4 (36.4)/4 (21.1)	8 (72.7)/10 (52.6)	6 (54.5)/8 (42.1)	0/3 (15.8)	0/0	0/0	SHV-1 (3/4), SHV-5 (1/0), CTX-M-1 (3/2), CTX-M-3 (1/1), CTX-M-15 (4/7), CMY-2 (0/3)	8/10
K. pneumoniae	44/18	33 (75.0)/7 (38.9)	39 (88.6)/12 (66.7)	37 (84.1)/4 (22.2)	17 (38.6)/2 (11.1)	2 (4.5)/0	0/0	0/0	SHV-1 (40/12),CTX-M-1 (11/0), CTX-M-3 (1/0), CTX-M-15 (25/3), CTX-M-22 (0/1), CMY-2 (2/0), DHA-1 (1/0)	38/8
K. oxytoca	7/9	5 (71.4)/5 (55.6)	6 (85.7)/1 (11.1)	5 (71.4)/2 (22.2)	3 (42.9)/2 (22.2)	2 (28.6)/1 (11.1)	0/0	0/0	SHV-1 (5/1), SHV-5 (1/0), CTX-M-1 (1/0), CTX-M-3 (1/0), CTX-M-15 (2/2), CTX-M-28 (1/0), CMY-2 (1/1), DHA-1 (1/0)	7/6
E. cloacae	7/3	5 (71.4)/1	1 (14.3)/1	3 (42.9)/1	2 (28.6)/1	1 (14.3)/0	0/0	0/0	SHV-1 (1/1), CTX-M-1 (1/0), CTX-M-15 (1/1), CTX-M-28 (1/0), CMY-2 (1/0)	4/1
C. freundii	3/2	2/0	1/0	2/0	0/0	0/0	0/0	0/0	SHV-1 (1/0), CTX-M-15 (2/0)	2/0
M. morganii	0/2	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Proteus spp.	5/5	2/2	0/0	4/1	3/1	1/1	0/0	0/0	CTX-M-1 (3/1), CTX-M-15 (1/0), CMY-2 (1/1)	4/2
P. aeruginosa	1/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
A. baumannii	10/3	5 (50.0)/2	0/0	2 (20.0)/1	2 (20.0)/1	-	6 (60.0)/1	0/0	CTX-M-1 (2/1)	5/2
Total	88/62	58 (65.9)/30 (48.4)	51 (58.0)/18 (29.0)	61 (69.3)/19 (30.6)	33 (37.5)/15 (24.2)	6 (6.8)/5 (8.1)	6/1	0/0	SHV-1 (49/18), SHV-5 (2/0), CTX-M-1 (21/4), CTX-M-3 (3/1), CTX-M-15 (35/13),CTX-M-22 (0/1), CTX-M-28 (2/0), CMY-2 (4/3), DHA-1 (2/0)	68/29

CTX: Ceftriaxone; *E. coli: Escherichia coli; K. pneumoniae: Klebsiella pneumoniae; K. oxytoca: Klebsiella oxytoca; E. cloacae: Enterobacter cloacae; C. freundii: Citrobacter freundii; M. morganii: Morganella morganii; P. aeruginosa: Pseudomonas aeruginosa; A. baumannii: Acinetobacter baumannii;* ESBL: Extended-spectrum beta-lactamase

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TABLE 2. Distribution of ESBL genotypes in inpatient and outpatient isolates

Types of beta-lactamases	*E. coli**		*K. pneumoniae**		*K. oxytoca*		*E. cloacae**		*A. baumannii*		Others*		Total*	
One type	In (10)	Out (14)	In (44)	Out (17)	In (7)	Out (9)	In (6)	Out (2)	In (10)	Out (3)	In (8)	Out (4)	In (88)	Out (62)
TEM	1	3					2		3	1	1	2	7	6
SHV	1	1	6	9		3		1			1		8	14
CTX-M												1		1
CMY-2		1												1
OXA-51									2				2	
Total	2	5	6	9		3	2	1	5	1	2	3	17	22
Two type														
TEM+SHV			1	4		3							1	7
TEM+CTX-M											1		1	
SHV+CTX-M	1		4	1									5	1
CTX-M+OXA-1	2	1					1	1			3		6	2
CTX-M+CMY-2		1												1
SHV+DHA-1					1								1	
SHV+CMY-2						1								1
TEM+CMY-2							1					1	1	1
TEM+OXA-51									3	1			3	1
Total	3	2	5	5	1	4	2	1	3	1	4	1	18	14
Three type														
CTX-M+SHV+OXA-1	1	1	5		1	2							7	3
TEM+CTX-M+OXA-1	3	3					1				1	1	5	4
TEM+CTX-M+CMY-2		1												1
TEM+SHV+CMY-2					1								1	
CTX-M+OXA-1+CMY-2											1		1	
Total	4	5	5		2	2	1				2	1	14	8
Three and more than three genes														
TEM+SHV+CTX-M+OXA-1	1	3	28	3	4		1						34	6
TEM+CTX-M+OXA-1+OXA-51									2	1			2	1
Total	1	3	28	3	4		1		2	1			36	7

*Three inpatient and 13 outpatient isolates were phenotypic-positive ESBL but did not show any type of beta-lactamases by PCR. *A. baumannii: Acinetobacter baumannii;* ESBL: Extended-spectrum beta-lactamase; CTX: Ceftriaxone; *E. coli: Escherichia coli; K. pneumoniae: Klebsiella pneumoniae; K. oxytoca: Klebsiella oxytoca; E. cloacae: Enterobacter cloacae*

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TABLE 3. MICs of various antibiotics for isolates which produce one and more than two type of beta-lactamases

Causative agent isolated	Setting	Number of isolates tested	MIC (mg/L) of antibiotics* (% of resistance isolates)										
			AMC	CZ	CXM	CAZ	CTX	CRO	FOX	FEP	IMI	MEM	GEN	CIP
Escherichia coli	Inpatient >2 genes	8	100	100	100	75.0	75.0	87.5	100	62.5	0	0	100	50
	Outpatients one gene	9	43.8	100	100	44.4	66.7	66.7	77.7	55.5	0	0	77.7	66.7
	Outpatient >2 genes	10	100	100	100	60.0	60.0	70.0	70.0	10.0	0	0	70.0	60.0
Klebsiella spp.	Inpatients one gene	6	50.0	83.3	53.3	50.0	50.0	83.3	100	50.0	0	0	83.3	66.7
	Inpatient >2 genes	45	100	100	97.8	82.2	84.4	86.7	95.6	53.3	0	0	75.6	55.6
	Outpatients one gene	12	50.0	100	100	75.0	66.7	58.3	83.3	66.7	0	0	83.3	50.0
	Outpatient >2 genes	14	100	100	100	85.7	64.3	78.6	57.1	28.6	0	0	78.6	57.1
Acinetobacter spp.	Inpatients one gene	6	100	100	100	100	83.3	83.3	100	66.7	83.3	16.7	66.7	66.7
	Inpatient >2 genes	5	100	100	100	80.0	80.0	100	100	100	100	40.0	100	60.0
Ostalo†	Inpatients one gene	7	100	100	71.4	42.9	14.3	42.9	100	14.3	0	0	71.4	42.9
	Inpatient >2 genes	10	100	100	90.0	80.0	90.0	80.0	70.0	70.0	0	0	60.0	80.0
	Outpatients one gene	12	100	100	100	75.0	50.0	100	83.3	25.0	8.3	8.3	100	41.7
	Outpatient >2 genes	5	100	100	80.0	80.0	60.0	80.0	80.0	20.0	40.0	20.0	100	60.0

*Amoxicillin/clavulanic, AMC, cefazolin, CZ, cefuroxime, CXM, ceftazidime, CAZ, cefotaxime, CTX, ceftriaxone, CRO, cefoxitin, FOX, cefepime, FEP, imipenem, IPM, meropenem, MEM, gentamicin, GEN, ciprofloxacin, CIP †Inpatients with one gene: *Citrobacter* spp. - 1; *E. coli* - 2; *Enterobacter* spp. - 3; *Pseudomonas* spp.-1; and >2 genes: *Citrobacter* spp. - 2; *Enterobacter* spp. - 4; *Proteus* spp. – 4. Outpatients with one gene: *Acinetobacter* spp. - 1; *Citrobacter* spp. - 2; *Enterobacter* spp. - 3; *Morganella morganii* - 2; *Proteus* spp. - 3; *Pseudomonas* spp. - 1; and with >2 genes: *Acinetobacter* spp. - 2; *Enterobacter* spp. - 1; *Proteus* spp. - 1. MIC: Minimum inhibitory concentration; *E. coli: Escherichia coli*

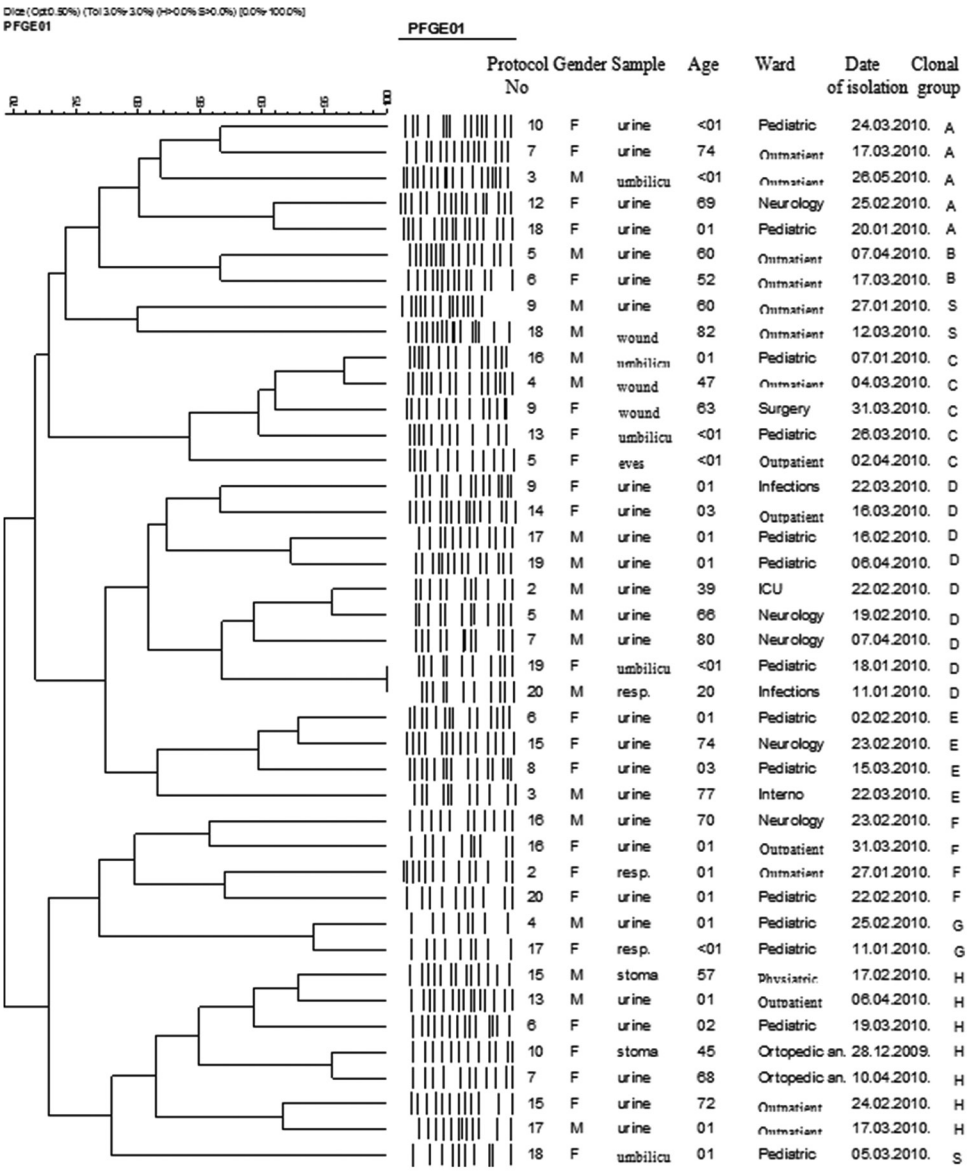

FIGURE 1. Dendograms showing the genetic relatedness of the 86 *Enterobacteriaceae isolates*. Eight (A-H) groups of *Klebsiella pneumoniae* were identified by pulsed-field gel electrophoresis (PFGE) typing using the 80% similarity (a). Four clones consisted of more than five inpatient and outpatient isolates. PFGE typing was not successful in 21 isolates. Six (A-F) groups of *Escherichia coli* were identified by PFGE typing using the 80% similarity (b). Three clones consisted of three isolates in each of the two outpatients and one inpatient isolates. PFGE typing was not successful in 12 isolates. Four (A-D) groups of *Proteus* spp. were identified by PFGE typing using the 80% similarity (c). Only one consisted of three inpatient isolates. PFGE typing was not successful in one isolate. Two (A and B) groups of Enterobacter cloacae were identified by PFGE typing using the 80% similarity (d). One clone consisted of six isolates: Three inpatient and three outpatient isolates. Two (A and B) groups of *Citrobacter freundii* were identified by pulsed field gel electrophoresis typing using the 80% similarity (e). (a) *K. pneumoniae*, (b) *E. coli*, (c) *Proteus* spp., (d) *E. cloacae*, (e) *C. freundii*. ICU - Intensive care unit.

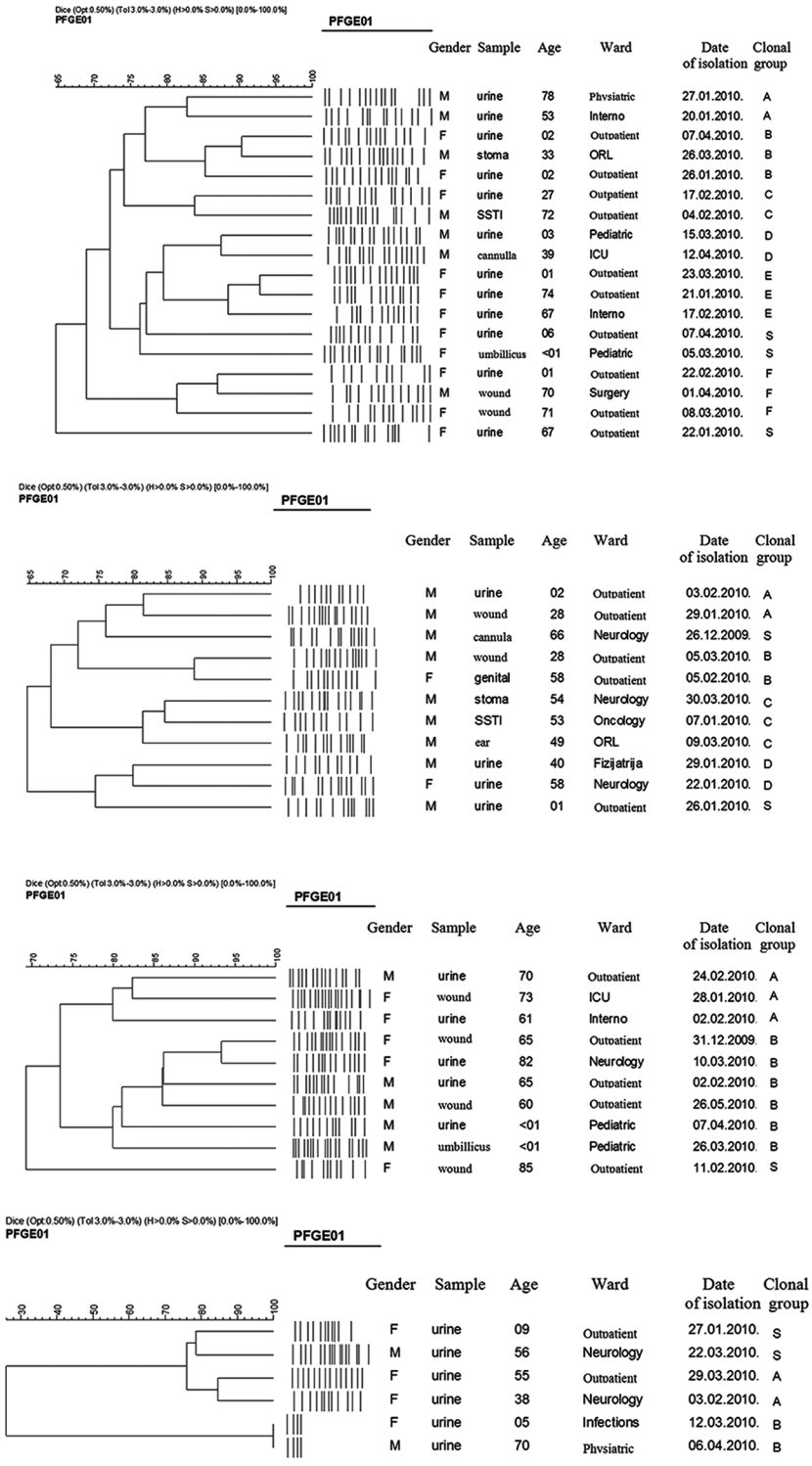

FIGURE 1. (Continued)

with the findings in Morocco, where *E. coli* was the most frequently noticed (23).

Isolates obtained in this study, which produced one type of beta-lactamases, showed lower prevalence of resistance to cefotaxime, ceftriaxone, and ceftazidime than isolates coproducing more than two types of beta-lactamases.

Several distinct PFGE profiles were detected among the inpatient and outpatient ESBL- positive strains, indicating that its predominance in our setting was not due to the spreading of a single clone, but rather due to the horizontal transfer of plasmids containing genes between different species of Enterobacteriaceae. In the hospital environment, under selection pressure, plasmids could be transferred between the patients and hospital personnel by hands (11,24). The possible explanation for this observation is the fact that ESBLs are encoded on plasmids and therefore could be easily transmitted as resistance gene elements for other antimicrobials, as well as from one organism to another (25).

There are many risk factors for colonization or infection with ESBL-producing isolates, such as long-term antibiotic exposure, prolonged intensive care unit stay, nursing home residency, severe illness, residence in an institution with frequent use of ceftazidime and other third-generation cephalosporin, and instrumentation or catheterization (25). Correct use of sterile gloves and systematic hand decontamination before and after visiting the patients and visitors' restriction are some of isolation measures (26).

Systematic surveillance of antimicrobial resistance which is the first step toward appropriate control of antibiotic usage does not exist in B and H. Further epidemiological surveillance studies are needed to provide useful information for prescription of antibiotics and their rational use. In addition to this, further studies are needed for a better evaluation of the epidemiology of ESBL-producing Gram-negative bacteria causing infections to develop effective prevention strategies aiming to control the spreading of infections.

CONFLICT OF INTEREST

There is no financial, personal, or academic conflict of interest.

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REFERENCES

1. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev 2005;18:657-86.
   https://doi.org/10.1128/CMR.18.4.657-686.2005.
2. Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: Toward the globalization of CTX-M. Clin Microbiol Rev 2013;26:744-58.
   https://doi.org/10.1128/CMR.00023-13.
3. Tacão M, Moura A, Correia A, Henriques I. Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. Water Res 2014;48:100-7.
   https://doi.org/10.1016/j.watres.2013.09.021.
4. Shahid M, Singh A, Sobia F, Rashid M, Malik A, Shukla I, et al. Bla(CTX-M), bla(TEM), and bla(SHV) in *Enterobacteriaceae* from north-Indian tertiary hospital: High occurrence of combination genes. Asian Pac J Trop Med 2011;4:101-5.
   https://doi.org/10.1016/S1995-7645(11)60046-1.
5. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G, et al. Prevalence of blaTEM, blaSHV and blaCTX-M genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from northeast India. Indian J Pathol Microbiol 2014;57:249-54.
   https://doi.org/10.4103/0377-4929.134698.
6. Shi H, Sun F, Chen J, Ou Q, Feng W, Yong X, et al. Epidemiology of CTX-M-type extended-spectrum beta-lactamase (ESBL)-producing nosocomial-*Escherichia coli* infection in China. Ann Clin Microbiol Antimicrob 2015;14(1):4.
   https://doi.org/10.1186/s12941-015-0063-7.
7. Rezai MS, Salehifar E, Rafiei A, Langaee T, Rafati M, Shafahi K, et al. Characterization of multidrug resistant extended-spectrum beta-lactamase-producing *Escherichia coli* among uropathogens of pediatrics in north of Iran. Biomed Res Int 2015;2015:309478.
   https://doi.org/10.1155/2015/309478.
8. Schmiedel J, Falgenhauer L, Domann E, Bauerfeind R, Prenger-Berninghoff E, Imirzalioglu C, et al. Multiresistant extended-spectrum β-lactamase-producing *Enterobacteriaceae* from humans, companion animals and horses in central Hesse, Germany. BMC Microbiol 2014;14:187. https://doi.org/10.1186/1471-2180-14-187.
9. El Sherif RH, Maamoun HA. Molecular characteristics of extended-spectrum betalactamases among gram-negative isolates collected in Cairo University Hospital. Comparative Clin Pathol 2013;22:733-9.
   https://doi.org/10.1007/s00580-012-1475-9.
10. Moland ES, Hanson ND, Black JA, Hossain A, Song W, Thomson KS, et al. Prevalence of newer beta-lactamases in gram-negative clinical isolates collected in the United States from 2001 to 2002. J Clin Microbiol 2006;44(9):3318-24.
    https://doi.org/10.1128/JCM.00756-06.

11. Yahya Mohsen SM, Hamzah HA, Imad Al-Deen MM, Baharudin R. Antimicrobial susceptibility of *Klebsiella pneumoniae* and *Escherichia coli* with extendedspectrum β-lactamase associated genes in Hospital Tengku Ampuan Afzan, Kuantan, Pahang. Malays J Med Sci 2016;23(2):14-20.

12. Ibrahimagić A, Bedenić B, Kamberović F, Uzunović S. High prevalence of CTX-M-15 and first report of CTX-M-3, CTX-M-22, CTX-M-28 and plasmid-mediated ampC beta-lactamase producing Enterobacteriaceae causing urinary tract infections in Bosnia and Herzegovina in hospital and community settings. J Infect Chemother 2015;21(5):363-9.

https://doi.org/10.1016/j.jiac.2015.01.003.

13. Seyedjavadi SS, Goudarzi M, Sabzehali F. Relation between *bla*TEM, *bla*SHV and *bla*CTX-M genes and acute urinary tract infections. J Acute Dis 2016;5(1):71-6.

https://doi.org/10.1016/j.joad.2015.07.007.

14. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 7th ed. Approved Standard M02-A11. Wayne: CLSI; 2012.

15. Tenover FC, Emery SL, Spiegel CA, Bradford PA, Eells S, Endimiani A, et al. Identification of plasmid-mediated ampC beta-lactamases in *Escherichia coli, Klebsiella* spp. and *Proteus* species can potentially improve reporting of cephalosporin susceptibility testing results. J Clin Microbiol 2009;47(2):294-9.

https://doi.org/10.1128/JCM.01797-08.

16. Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA. New delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli, Canada*. Emerg Infect Dis 2011;17(1):103-6.

https://doi.org/10.3201/eid1701.101358.

17. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 2010;65:490-5.

https://doi.org/10.1093/jac/dkp498.

18. Pasanen T, Jalava J, Horsma J, Salo E, Pakarinen M, Tarkka E, et al. An outbreak of CTX-M-15-producing *Escherichia coli, Enterobacter cloacae*, and *Klebsiella* in children´s hospital in Finland. Scand J Infect Dis 2014;46:225-30.

https://doi.org/10.3109/00365548.2013.857045.

19. Kaufman ME. Pulsed-field gel electrophoresis. In: Woodfor N, Johnsons A, editorrs. Molecular Biology. Protocols and Clinical Applications. 1st ed. New York, Totowa: Humana Press Inc.; 1998. p. 33-51.

https://doi.org/10.1385/0-89603-498-4:33.

20. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 1995;33(9):2233-9.

21. Wienke M, Pfeifer Y, Weissgerber P, Marschal M, Autenrieth IB, Gröbner S, et al. *In vitro* activity of tigecycline and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from a university hospital in south-western Germany. Chemotherapy 2012;58(3):241-8.

https://doi.org/10.1159/000339488.

22. Rakotonirina HC, Garin B, Randrianirina F, Richard V, Talarmin A, Arlet G, et al. Molecular characterization of multidrug-resistant extended-spectrum β-lactamase-producing *Enterobacteriaceae* isolated in Antananarivo, Madagascar. BMC Microbiol 2013;13:85.

https://doi.org/10.1186/1471-2180-13-85.

23. El bouamri MC, Arsalane L, Zerouali K, Katfy K, El kamouni Y, Zouhair S. Molecular characterization of extended spectrum β-lactamase-producing Escherichia coli in a university hospital in Morocco, North Africa. African J of Urology 2015;21:161-6.

https://doi.org/10.1016/j.afju.2015.02.005.

24. Gulamber C, Altindis M, Kalayci R, Aktepe OC. Molecular characterization of nosocomial CTX-M type β-lactamase producing Enterobacteriaceae from University Hospital in Turkey. Afr J Microbiol Res 2012;6:5552-7.

25. Ibrahim ME, Bilal NE, Magzoub MA, Hamid ME. prevalence of extended-spectrum βlactamases-producing *Escherichia coli* from hospitals in Khartoum State, Sudan. Oman Med J 2013;28:116-20.

https://doi.org/10.5001/omj.2013.30.

26. Rubio-Perez I, Martin-Perez E, Garcia DD, Calvo ML, Barrera EL. Extended-spectrum beta-lactamase-producing bacteria in a tertiary care hospital in Madrid: Epidemiology, risk factors and antimicrobial susceptibility patterns. Emerg Health Threats J 2012;5.

https://doi.org/10.3402/ehtj.v5i0.11589.