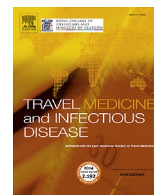
Travel Medicine and Infectious Disease 14 (2016) 572–576

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevierhealth.com/journals/tmid



Original article

Investigation of carbapenem resistance and the first identification of *Klebsiella pneumoniae* carbapenemase (KPC) enzyme among *Escherichia coli* isolates in Turkey: A prospective study

Mert Ahmet Kuskucu [a], Asiye Karakullukcu [a, *], Mailihaba Ailiken [a], Barıs Otlu [b], Bilgul Mete [c], Gokhan Aygun [a]

[a] Cerrahpasa Medical School of Istanbul University, Department of Medical Microbiology, Istanbul, Turkey
[b] Inonu University Faculty of Medicine, Department of Medical Microbiology, Malatya, Turkey
[c] Cerrahpasa Medical School of Istanbul University, Department of Infectious Diseases, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 14 October 2016
Received in revised form
18 November 2016
Accepted 21 November 2016
Available online 23 November 2016

Keywords:
Escherichia coli
Carbapenem resistance
Carbapenemase
Klebsiella pneumoniae carbapenemase
OXA-48

ABSTRACT

Background: The aim of this study was to determine the presence of carbapenem resistance and carbapenemase production in *Escherichia coli* isolates from clinical samples in Turkey.
Methods: The prospective study included a total of 4.052 *Escherichia coli* isolates collected from patients admitted to a hospital from March 2011 to May 2012. We used ertapenem disc for screening carbapenemase production, and the confirmation was performed by using Etest. The resistance mechanisms and genetic relatedness of the carbapenem resistant strains were investigated by using PCR (polymerase chain reaction) and pulsed-field gel electrophoresis (PFGE), respectively.
Results: Among the 4.052 *E. coli* isolates, 24 (0.59%) were found to be carbapenem resistant. Of these, only 5 isolates were positive for OXA-48 and 2 isolates were positive for *Klebsiella pneumoniae* carbapenemase (KPC)-2. The KPC-2 producing *E. coli* strains (n = 2) were both isolated from the same patient. The bla_{KPC} genes were confirmed using DNA sequence analysis. The genetic relationship between the 24 *E. coli* strains studied by PFGE revealed that the strains were genetically unrelated.
Conclusions: This article confirms, to our knowledge for the first time, the detection of KPC-2-producing *E. coli* in Turkey, with OXA-48 being the most frequent carbapenemase in the study.
© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Infection caused by multidrug-resistant *Escherichia coli* and other *Enterobacteriaceae* has become an important clinical problem associated with reduced therapeutic possibilities. Carbapenems (imipenem, meropenem, and ertapenem) are considered to be the last line of defense against these bacteria, but increasing spread of mobile genetic elements carrying carbapenemase genes such as bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{OXA-48} have caused the loss of clinical efficacy of carbapenems [1]. Carbapenem resistance in *Enterobacteriaceae* can be caused by a variety of mechanisms including the presence of carbapenemases or other *beta*-lactamases in combination with porin deficiency [2]. Especially, the occurrence of an outer-membrane porin deficiency and the expression of plasmid-mediated AmpC beta-lactamases were reported to be responsible for carbapenem resistance in *E. coli* [3]. Carbapenemase-producing *E. coli* has been relatively less reported, but an increase of carbapenem-resistant *Klebsiella pneumoniae* has been observed [4]. The first case of carbapenem-resistant *E. coli* strain had been described from Greece with the decreased carbapenem susceptibility and production of a metallo-beta-lactamase, VIM (Verona intergron-encoded metallo-beta-lactamase) [5]. *Klebsiella pneumoniae* carbapenemase (KPC) enzymes are most frequently detected in *K. pneumoniae*, but they are also being increasingly detected in *E. coli.* The first report of KPC-2 in *E. coli* was in 2006 [6]. During the following years, KPC-type enzymes continued to be reported in *E. coli* from United States, Israel, France and China [7–11]. A single report has been described in *E. coli* from

* Corresponding author. Cerrahpasa Medical School of Istanbul University, Department of Medical Microbiology, Cerrahpasa Street, 34098 Istanbul, Turkey. Tel.: +90 2124143000, +90 2124122754, +90 5375783472; fax: +90 2126322122.
E-mail addresses: kuskucum@gmail.com (M.A. Kuskucu), asiyekarakullukcu@gmail.com (A. Karakullukcu), mailihaba.ailiken@gmail.com (M. Ailiken), botlu@yahoo.com (B. Otlu), bigimete@istanbul.edu.tr (B. Mete), gokhanaygun67@yahoo.com (G. Aygun).

http://dx.doi.org/10.1016/j.tmaid.2016.11.006
1477-8939/© 2016 Elsevier Ltd. All rights reserved.

United States, involving KPC-3 acquisition in a patient during imipenem therapy [12]. The first report of KPC-type carbapenemase from Turkey was in *K. pneumonia* [13]. Then two isolates producing *K. pneumonia* carbapenemase were also reported in *K. pneumonia* from Turkey [14]. The OXA-48 carbapenemase was first identified in *Enterobacteriaceae* in Turkey in 2001, and now OXA-48 is known to be endemic in Turkey [15]. Over the years, OXA-48 and OXA-48-like carbapenemases are also increasingly reported in *E. coli* from Germany, France, Israel, Senegal and Japan [16–20].

We undertook a prospective study to evaluate the presence of carbapenem resistance in clinical *E. coli* isolates, and identify the epidemiological and genetic relatedness as well as the enzymatic mechanisms leading to carbapenem resistance in this species. To our best knowledge, we reported the first KPC- producing *E. coli* in Turkey.

2. Materials and methods

2.1. Bacterial strains

The study was conducted at Cerrahpasa Medical School, a 1.300-bed tertiary care teaching hospital in Turkey. We investigated the presence of carbapenem resistance and carbapenemase production in clinical *E. coli* isolates collected from patients admitted to a hospital in Turkey from March 2011 to May 2012. If the samples from the same patient had been sent to the laboratory more than 7 days interval, the *E. coli* strains were considered as different isolates. Only 6 such isolates from the 3 patients were recovered in the study. All isolates were identified by using an automated VITEK 2 System (bioMerieux, France).

2.2. Antimicrobial drug susceptibility testing

Antibiotic susceptibility was determined by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. The following antimicrobial agents were tested: ampicillin, amoxicillin-clavulanic acid, cefuroxime, cefotaxime, ceftazidime, cefepime, gentamicin, netilmicin, amikacin, ciprofloxacin, piperacilin-tazobactam, trimethoprim-sulfamethoxazole, ertapenem, imipenem, and meropenem. Double-disk synergy tests were performed for extended-spectrum β-lactamase (ESBL) detection as described previously [22]. We used ertapenem disc (10 μg/ml; Oxoid Ltd, Basingstoke, UK) for screening carbapenemase production based on the CLSI recommandations, and the confirmation was performed by using ertapenem, imipenem and meropenem E-test strips (Liofilchem, Italy). All break points were applied according to the CLSI guidelines [21]. Quality control was performed by using *E. coli* ATCC 25922 reference strain.

2.3. Screening of carbapenemase genes and sequencing of bla_{KPC}

The carbapenem-resistant *E. coli* strains were tested for the detection of bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, $bla_{OXA\text{-}48}$ carbapenemase genes, and genes encoding other β-lactamases ($bla_{CTX\text{-}M}$) by using PCR (polymerase chain reaction), as described previously [23,24]. 16s rRNA was also used as an internal control for excluding false negative results due to PCR inhibition, and negative controls were included each lots of PCR setups for excluding false positive results due to cross contamination. Amplifications were performed by using the primers listed in Table 1. Positive PCR product for bla_{KPC} gene was sequenced by using the amplification primers. The sequencing approach of bi directional DNA was performed by using dye terminator reaction with an automated system (ABI PRISM 310 Genetic Analyser, Applied Biosystems). The obtained sequence data were edited by using DNASTAR software (DNASTAR, Madison,WI). A BLAST (Basic Local Alignment and Search Tool) search was performed with National Center for Biotechnology Information (NCBI) database.

2.4. PFGE

Epidemiologic relatedness of carbapenem-resistant *E. coli* strains was studied with using PFGE (pulsed-field gel electrophoresis) as described previously [25]. PFGE was conducted by macrorestriction of chromosomal DNA with *Xba*I and separation of restriction fragments using CHEF DRII PFGE system (Bio-Rad Laboratories, Nazareth, Belgium). Migration of DNA fragments was normalized by using an appropriate mass marker, and computer-assisted analysis of PFGE patterns was conducted by using Bionumerics software (version 6.0; Applied Maths, Sint-Martens-Latem, Belgium). PFGE types were defined on the basis of DNA banding patterns in accordance with criteria defined by Tenover et al. [26].

3. Results

A total of 4.052 *E. coli* strains were isolated from the different clinical samples of patients during the period. 852 (21%) of the 4.052 *E. coli* isolates were found positive for ESBL production. Out of the 4.052 *E. coli* strains, 24 (0.59%) were found resistant to ertapenem, but all isolates were found susceptible to meropenem and imipenem with the disc diffusion method. The 24 strains isolated from 23 different patients were multidrug resistant, with different antibiotic susceptibilities. MICs of carbapenems showed

Table 1
Primers used for beta-lactamase detection.

β-lactamases	Primer abbreviation	Primer sequence	Tm[a] (°C)	Gene bank	Position	Product (bp)
bla_{VIM}[b]	Pan_VIM_Fw	TTCTCGCGGAGATTGARAAGC	54	JN819277	219–239	264
	Pan_VIM_Rev	TTGTCGGYYGAATGCGCAGC			483–464	
bla_{IMP}[b]	Pan_IMP_Fw	GGAATAGAGTGGCTTAAYTCTC	50	GU207399	372–393	188
	Pan_IMP_Rev	ARCCAAACYACTASGTTATC			560–543	
$bla_{OXA\text{-}48}$[b]	OXA-48_Fw	GCGTGTATTAGCCTTATCGGC	52	JN626286	5518–5537	722
	OXA-48_Rev	RGGCATATCCATATTCATCGC			6240–6220	
$bla_{NDM\text{-}1}$[b]	NDM_Fw	GGGCAGTCGCTTCCAACGGT	55	JQ734687	212–231	475
	NDM_Rev	GTAGTGCTCAGTGTCGGCAT			687–668	
bla_{KPC}[b]	KPC_Fw	GCTGTCTTGTCTCTCATGGCC	55	JQ867396	394–414	836
	KPC_Rev	AATCCCTCGAGCGCGAGTCTA			1230–1210	
$bla_{CTX\text{-}M}$[b]	CTXM_Fw	ATCTGACGCTGGGTAAAGC	50	JQ686201	695–713	162
	CTXM_Rev	ATATCGTTGGTGGTGCCATA			857–838	

[a] Tm, Temperature.
[b] VIM, Verona integron-encoded metallo-beta-lactamase; IMP, Imipenemase; OXA-48, Oxacillinase-48; NDM, New Delhi metallo-beta-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; CTX-M, Cefotaxime-hydrolyzing beta-lactamase.

that 7 of the 24 isolates were ertapenem susceptible, two were imipenem resistant, and one were meropenem resistant. This condition can be interpreted as the decreased level of resistance or relatively lower specificity of disc diffusion test for carbapenems.

Based on the DNA sequence analysis, 21 of the 24 *E. coli* strains were found positive for $bla_{CTX-M-15}$. All of the 24 *E. coli* strains were negative for bla_{NDM}, bla_{VIM}, and bla_{IMP} carbapenemase genes, but the 5 and 2 isolates were positive for bla_{OXA-48} and bla_{KPC-2} genes, respectively. KPC positive *E. coli* strains ($n = 2$) were both isolated from the same patient. The obtained bla_{KPC} gene sequence was 100% identical to the nucleotide sequence of KPC-2 gene (GenBank accession number: JQ867396). The MIC values of carbapenems tested for carbapenem-resistant *E. coli* isolates, and the results of PFGE and PCR for *bla* genes are shown in Table 2.

The genetic relationship between the 24 strains studied by PFGE revealed eighteen different *Xba*I endonuclease-restricted DNA profiles, with band difference greater than seven, indicating that these *E. coli* strains were genetically unrelated, and the clonal spread was not responsible for the emergence of carbapenem-resistance in *E. coli* isolates. No major cluster was found. In the genotype one, 18. isolate carried the bla_{OXA-48} and $bla_{CTX-M-15}$ genes and was resistant to gentamicin, amoxicillin-clavulanic acid, and piperacillin-tazobactam antibiotics, but the 20. isolate was negative for bla_{OXA-48} and $bla_{CTX-M-15}$ genes and was susceptibility to gentamicin, amoxicillin-clavulanic acid, and piperacillin-tazobactam antibiotics. There may have been transformation of plasmid DNA from the 18. sample to the 20. sample. Plasmid DNA analysis of the *E. coli* strains should be done to explain this condition. The KPC-2 positive *E. coli* isolates (3. and 6. isolates) from the same patinets were clonally related, exhibiting identical restriction patterns. The results of PFGE and DNA sequence analysis are shown in Fig. 1.

4. Discussion

Carbapenem resistance has emerged recently, and carbapenem-resistant *Enterobacteriaceae* today are considered as an urgent threat [27]. For this reason, advanced studies which will provide some data about the epidemiology of resistance and the useful methods to detect carbapenem-resistant isolates are still certainly needed.

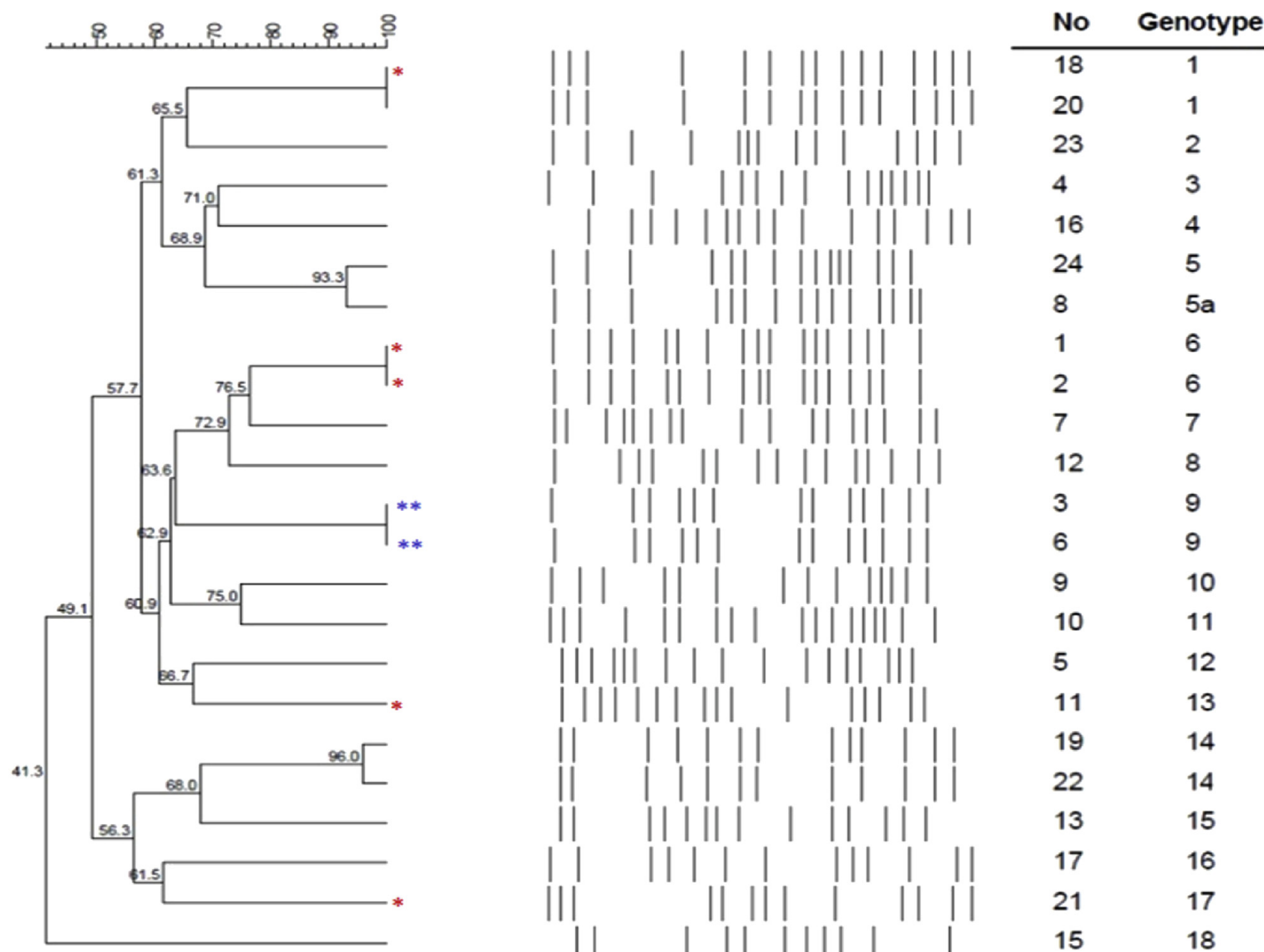
At the beginning of the 2012, ertapenem has been recommended for detecting carbapenemase production in *Enterobacteriacea* [21]. In the study, we used the ertapenem disc for screening carbapenemase production, and found only ertapenem resistance by disc diffusion. Although all isolates with carbapenemase genes were resistant or intermediate to ertapenem, only two of the OXA-48 positive strains were resistance to imipenem and one were resistance to meropenem according to the E-test results. Ertapenem seems to be a sensitive agent in screening carbapenemases, which OXA-48 and KPC-2 are investigated, and it can be useful in centers where molecular tests are not available. In contrast to our study results, ertapenem is not advised as an indicator of carbapenem susceptibility in recent guidelines because isolates with AmpC/ESBL and decreased permeability have higher MICs for ertapenem than for imipenem or meropenem [28]. In our study, only 7 of the 24 (29.1%) carbapenem-resistant *E. coli* isolates were found positive for carbapenemase genes. This condition may be suggesting that OmpC and/or OmpF deficiency combined with AmpC can be the responsible mechanism for the development of carbapenem resistance in *E. coli* in Turkey. In a previous study, researchers investigated a carbapenem-resistant *E. coli* strain from a patient with pertonitis, and they found that the carbapenem resistance occured because of a combination of OmpC loss and CTX-M production [29].

The KPCs are plasmid-encoded enzymes mostly reported in *K. pneumoniae* from the United States. The first KPC-positive

Table 2
MIC values of carbapenems tested for carbapenem-resistant *Escherichia coli* isolates, and results of PFGE and PCR analysis of *bla* genes.

Sample No	MICs of carbapenems (μg/m)			Beta-lactamases						Genotype
	Ertapenem	Meropenem	Imipenem	bla_{OXA-48}	bla_{KPC}	bla_{NDM-1}	bla_{VIM}	bla_{IMP}	bla_{CTX-M}	
1	32	4[c]	2	**Positive**[b]	Negative	Negative	Negative	Negative	Positive	6
2	2	2	4[c]	**Positive**[b]	Negative	Negative	Negative	Negative	Positive	6
3	32	1,5	3	Negative	**Positive**[b]	Negative	Negative	Negative	Positive	9
4	0,75	0,38	1,5	Negative	Negative	Negative	Negative	Negative	Positive	3
5	8	0,25	0,75	Negative	Negative	Negative	Negative	Negative	Positive	12
6	32	1,5	3	Negative	**Positive**[b]	Negative	Negative	Negative	Positive	9
7	0,032[a]	0,016	0,19	Negative	Negative	Negative	Negative	Negative	Positive	7
8	0,047[a]	0,032	0125	Negative	Negative	Negative	Negative	Negative	Positive	5a
9	0,19[a]	0,016	0125	Negative	Negative	Negative	Negative	Negative	Positive	10
10	2	0,25	0,38	Negative	Negative	Negative	Negative	Negative	Positive	11
11	0,75	0,023	0,25	**Positive**[b]	Negative	Negative	Negative	Negative	Negative	13
12	0,25[a]	0,016	0,25	Negative	Negative	Negative	Negative	Negative	Positive	8
13	4	0,19	0,19	Negative	Negative	Negative	Negative	Negative	Positive	15
14	0,75	0,125	0,75	Negative	Negative	Negative	Negative	Negative	Positive	Non typable[d]
15	0,25[a]	0,032	0,19	Negative	Negative	Negative	Negative	Negative	Positive	18
16	2	0,19	0,19	Negative	Negative	Negative	Negative	Negative	Positive	4
17	0,25[a]	0,064	0125	Negative	Negative	Negative	Negative	Negative	Positive	16
18	8	2	4[c]	**Positive**[b]	Negative	Negative	Negative	Negative	Positive	1
19	0,25[a]	0,064	0125	Negative	Negative	Negative	Negative	Negative	Positive	14
20	1	0,5	0,5	Negative	Negative	Negative	Negative	Negative	Negative	1
21	2	0,25	2	**Positive**[b]	Negative	Negative	Negative	Negative	Negative	17
22	4	0,5	0,25	Negative	Negative	Negative	Negative	Negative	Positive	14
23	2	0,5	0,75	Negative	Negative	Negative	Negative	Negative	Positive	2
24	4	1	1	Negative	Negative	Negative	Negative	Negative	Positive	5

PFGE, Pulsed-field gel electrophoresis; PCR, Polymerase chain reaction, MIC, minimum inhibitory concentration.
[a] Ertapenem susceptible isolates, MICs$\leq$ 0.5 μg/m.
[b] OXA-48 and KPC-2-positive isolates.
[c] Imipenem and meropenem resistant isolates, MICs$\geq$ 4 μg/m.
[d] 14.sample was not typable by PFGE.



*OXA-48-positive isolates.

**KPC-2-positive isolates.

Fig. 1. Results of pulsed-field gel electrophoresis and DNA sequence analysis for the 24 carbapenem-resistant *Escherichia coli* isolates.

K. pneumoniae infection outside USA was reported in 2005 from France, the first outbreak was from Israel. Besides USA, Greece is considered as another country with epidemicity of KPC-producing bacteria. Other sporadic reports of KPC-positive strains were generally linked with travel histories to USA, Israel or Greece [2]. KPC-2-producing *K. pneumoniae* has recently been reported from Turkey [13,14], whereas KPC-2-producing *E. coli* has not been reported. This report confirms, to our knowledge for the first time, the arrival of KPC-2-producing *E. coli* in Turkey. The first KPC-positive strains from Turkey in *Escherichia coli* were isolated from the blood cultures of a Turkish patient with a long stay in intensive care unit.

Turkey has been accepted is the main reservoir for OXA-48 producers [30–32]. In our study, OXA-48 was also the most frequent carbapenemase, but there is no homogeneity among OXA-48 positive strains in antibiotic resistance profiles and carbapenem MICs. Continuous surveillance and molecular characterization of OXA-48 producers are needed to better understand the transmission pathways, and to establish proper infection control policies.

VIM- and IMP-type enzymes are known to be endemics in Greece, Taiwan, and Japan, but at the same time outbreaks and single reports of VIM and IMP producers have been reported in many other countries. After the description of NDM-1 in *K. pneumoniae*, this gene began to be reported in *E coli* generally in the strains isolated from patients with travel history to Indian subcontinent [2]. Despite the recent studies from Turkey with NDM-type carbapenamase strains in *Enterobacterecae* [14,33], there was no NDM-type carbapenemase in *E. coli* isolates in our study.

In conclusion, we report here the presence of carbapenem resistance in *E. coli* isolates from the clinical samples and the first identification of the KPC-2-producing *E. coli* isolates in Turkey. OXA-48-type carbapenemase was the most frequent, as it is generally reported in Turkey, and it is probable that KPC-type carbapenemase may be present more frequently in Turkey, especially in *K. pneumonia,* but it is not been monitored routinely therefore it can not be detected. There is a need for more studies to understand better the epidemiology of resistance factors in *E. coli* in Turkey.

Acknowledgments

The study was partially funded by a project (no: 17546/2012)

from the Research Found of Istanbul University and approved by the Ethics committees of Cerrahapasa Medical Faculty, Istanbul, Turkey. The preliminary data belongs this work was presented at the XXXV. Turkish Microbiology Congress, 3–7 November 2012, Kuşadası, Turkey (Oral presentation, hall A, no: S.4.1).

References

[1] Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 2011;17:1791–8.
[2] Patel JB, Rasheed JK, Kitchel B. Carbapenemases in *Enterobacteriaceae*: activity, epidemiology, and laboratory detection. Clin Microbiol News 2009;31(8):55–62.
[3] Stapleton PD, Shannon KP, French GL. Carbapenem resistance in *Escherichia coli* associated with plasmid-determined CMY-4 beta-lactamase production and loss of an outer membrane protein. Antimicrob Agents Chemother 1999;43:1206–10.
[4] Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 2009;9:228–36.
[5] Miriagou V, Tzelepi E, Gianeli D, Tzouvelekis LS. *Escherichia coli* with self-transferable multi-resistant plasmid coding for metallo-β-lactamase VIM-1. Antimicrob Agents Chemother 2003;47:395–7.
[6] Navon-Venezia S, Chmelnitsky I, Leavitt A, Schwaber MJ, Schwartz D, Carmeli Y. Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. Antimicrob Agents Chemother 2006;50(9):3098–101.
[7] Chen LH, Petersen E. The era of antibiotic-resistant bacteria—Time for political, travel medicine and healthcare decisions. Travel Med Infect Dis 2015 Jul-Aug;13(4):279–80. http://dx.doi.org/10.1016/j.tmaid.2015.07.001.
[8] Bratu S, Brooks S, Burney S, Kochar S, Gupta J, Landman D, et al. Detection and spread of *Escherichia coli* possessing the plasmid-borne carbapenemase KPC-2 in brooklyn, NewYork. Clin Infect Dis 2007;44:972–5.
[9] Goren MG, Navon-Venezia S, Chmelnitsky I, Carmeli Y. Carbapenem-resistant KPC-2-producing *Escherichia coli* in a Tel Aviv Medical center 2005 to 2008. Antimicrob Agents Chemother 2010;54:2687–91.
[10] Petrella S, Ziental-Gelus N, Mayer C, Renard M, Jarlier V, Sougakoff W. Genetic and Structural Insights into the dissemination potential of the extremely broad-spectrum class a β-lactamase KPC-2 identified in an *Escherichia coli* strain and an *Enterobacter cloacae* strain isolated from the same patient in France. Antimicrob Agents Chemother 2008;52:3725–6.
[11] Cai JC, Zhou HW, Zhang R, Chen GX. Emergence of *Serratia marcescens, Klebsiella pneumoniae* and *Escherichia coli* isolates possessing the plasmid-mediated carbapenem-hydrolyzing β-lactamase KPC-2 in intensive care units of a chinese hospital. Antimicrob Agents Chemother 2008;52:2014–8.
[12] Hong T, Smith Moland E, Abdalhamid B, Hanson ND, Wang J, Sloan C, et al. *Escherichia coli*: development of carbapenem resistance during therapy. Clin Infect Dis 2005;40:84–6.
[13] Labarca J, Poirel L, Ozdamar M, Turkoglü S, Hakko E, Nordmann P. KPC-producing *Klebsiella pneumoniae*, finally targeting Turkey. New Microbes New Infect 2014;2(2):50–1.
[14] Poirel L, Yilmaz M, Istanbullu A, Arslan F, Mert A, Bernabeu S, et al. Spread of NDM-1-producing *Enterobacteriaceae* in a neonatal intensive care unit in Istanbul, Turkey. Antimicrob Agents Chemother 2014;58(5):2929–33.
[15] Poirel L, Heáritier C, Tolun V, Nordman P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2004;48:15–22.
[16] Pfeifer Y, Schlatterer K, Engelmann E, Schiller RA, Frangenberg HR, Stiewe D, et al. Emergence of OXA-48-carbapenemase-producing *Enterobacteriaceae* in German hospitals. Antimicrob Agents Chemother 2012;56:2125–8.
[17] Poirel L, Bernabeau S, Fortineau N, Podglajen I, Lawrence C, Nordmann P. Emergence of OXA-48-Producing *Escherichia coli* clone ST38 in France. Antimicrob Agents Chemother 2011;55:4937–8.
[18] Goren MG, Chmelnitsky I, Carmeli Y, Navon-Venezia S. Plasmid-encoded OXA-48 carbapenemase in *Escherichia coli* from Israel. J Antimicrob Chemother 2011;66:672–3.
[19] Moquet O, Bouchiat C, Kinana A, Seck A, Arouna O, Bercion R, et al. Class D OXA-48 carbapenemase in multidrug-resistant enterobacteria. Senegal Emerg Infect Dis 2011;7:143–4.
[20] Nagano N, Endoh Y, Nagano Y, Toyama M, Matsui M, Shibayama K, et al. First report of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in Japan from a patient returned from Southeast Asia. Jpn J Infect Dis 2013;66:79–81.
[21] Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial susceptibility testing, twenty-second informational supplement. Wayne, PA: CLSI M100-S22-U; 2011.
[22] Peterson DL, Bonomo RA. Extended-spectrum β-lactamase: a clinical update. Clin Microbiol Rev 2005;18:657–86.
[23] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119–23.
[24] Ojdana D, Sacha P, Wieczorek P, Czaban S, Michalska A, Jaworowska J, et al. The occurrence of *bla*CTX-M, *bla*SHV, and blaTEM genes in extended-spectrum *β*-lactamase positive strains of Klebsiella pneumoniae, Escherichia coli, and Proteus mirabilis in Poland. Int J Antibiot 2014;2014:7. http://dx.doi.org/10.1155/2014/935842. Article ID 935842.
[25] Durmaz R, Otlu B, Koksal F, Hosoglu S, Ozturk R, Ersoy Y, et al. The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of *Acinetobacter baumannii, Escherichia coli* and *Klebsiella spp*. Jpn J Infect Dis 2009;62(5):372–7.
[26] 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;33:2233–9.
[27] Antibiotic resistance threats in the United States. Atlanta: Centers for Disease Control and Prevention (CDC); 2013 April 23. 2016 May (date cited), http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf.
[28] Woodford N, Dallow JW, HillR L, Palepou MF, Pike R, Ward ME, et al. Ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the UK to a reference laboratory. Int J Antimicrob Agents 2007;29:456–9.
[29] Lartigue MF, Poirel L, Poyart C, Reglier-Poupet H, Nordmann P. Ertapenem resistance of *Escherichia coli*. Emerg Infect Dis 2007;13:315–7.
[30] Gülmez D, Woodford N, Palepou MFI, Mushtaq S, Metan G, Yakupogullari Y, et al. Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from Turkey with OXA-48-like carbapenemases and outer membrane protein loss. Int J Antimicrob Ag 2008;31:523–6.
[31] Kilic A, Aktas Z, Bedir O, Gumral R, Bulut Y, Stratton C, et al. Identification and characterization of OXA-48 producing, carbapenem-resistant *Enterobacteriaceae* isolates in Turkey. Ann Clin Lab Sci 2011;41:161–6.
[32] Nazik H, Ongen B, Ilktac M, Aydin S, Kuvat N, Sahin A, et al. Carbapenem resistance due to *bla* (OXA-48) among ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in a university hospital, Turkey. Southeast Asian J Trop Med Public Health 2012;43:1178–85.
[33] Poirel L, Ozdamar M, Ocampo-Sosa AA, Turkoglu S, Guney Ozer U, Nordmann P. NDM-1-producing *Klebsiella pneumoniae* now in Turkey. Antimicrob Agents Chemother 2012;56:2784–5.