Nota breve

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Isolation of the first metallo-β-lactamase producing *Klebsiella pneumoniae* in Lebanon

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Introduction. A 58 year-old man was admitted to the Saint Joseph Hospital-Raymond and Aida Najjar polyclinic in Beirut on July 17, 2007 to undergo surgery for a moderately differentiated colonic adenocarcinoma (T3N0). Following several discharges and re-admissions, an extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* susceptible to imipenem was isolated. The patient was put on imipenem and metronidazole. Three weeks later, imipenem (IMP) resistant *Klebsiella pneumonia*e was isolated.

Methods and results. The antimicrobial susceptibility profile of the imipenem-resistant *Klebsiella pneumonia*e strain and related minimum inhibitory concentrations of antibiotics were determined. Hydrolysis of IMP was evaluated and production of metallo-β-lactamase (MBL) was detected by a double disk-synergy test, ethylene diamine tetraacetic acid (EDTA) inhibited the imipenemase activity, whereas clavulanate and tazobactam did not, this suggesting the production of a metallo-β-lactamase. Isoelectric focusing analysis was performed and indicated the presence of a cefotaximase (blaCTX-M-15). Polymerase chain reaction (PCR) was used and detected the presence of blaIMP-1 and blaCTX-M genes.

Conclusions. During the last decade, many hospital outbreaks caused by ESBL-producing Enterobacteriaceae spp. have been reported in Lebanon. To our knowledge, this is the first report of a clinical isolate of *K. pneumonia*e producing an MBL in Lebanon.

Key words:
Metallo-β-lactamase. *Klebsiella pneumoniae*. Extended spectrum beta-lactamase (ESBL). Resistance. Carbapenemases. BlaIMP-1. BlaCTX-M-15. Lebanon.

Rev Esp Quimioter 2008;21(2):123-126

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Aislado de la primera *Klebsiella pneumoniae* productora de metalo-betalactamasa en Líbano

Introducción. El 17 de julio de 2007 un varón de 58 años de edad fue ingresado en Hospital de Saint Joseph-Raymond and Aida Najjar Polyclinic de Beirut para someterse a una intervención quirúrgica por un adenocarcinoma moderadamente diferenciado de colon (T3N0). Después de varias altas y rehospitalizaciones se aisló una *Escherichia coli* productora de betalactamasa de espectro extendido y sensible al imipenem (IMP). El paciente fue tratado con imipenem y metronidazol. Una semana más tarde se efectuó un cultivo en el que se determinó *Klebsiella pneumoniae* resistente al imipenem.

Métodos y resultados. Se determinó el perfil de sensibilidad antimicrobiana de la cepa de *Klebsiella pneumoniae* resistente al imipenem y las concentraciones mínimas inhibitorias de los antibióticos. Se evaluó la hidrólisis del IMP y se detectó la producción de metalo-betalactamasa (MBL) mediante el ensayo de sinergia con doble disco. El ácido etilendiaminotetraacético (EDTA) inhibió la actividad de la imipenemasa, mientras que clavulanato y tazobactam no, lo que indica la producción de metalo-betalactamasa. Se efectuó un análisis por enfoque isoeléctrico que indicó la presencia de cefotaximasa (blaCTX-M-15). Para detectar la presencia de los genes blaIMP-1 y blaCTX-M se empleó la reacción en cadena de polimerasa *(polymerase chain reaction* [PCR]).

Conclusiones. Durante la última década se han documentado muchos brotes hospitalarios causados por especies de enterobacterias productoras de ESBL en Líbano. A nuestro entender éste es el primer informe de una cepa clínica de *K. pneumoniae* productora de MBL en Líbano.

Palabras clave:
Metalo-betalactamasas. *Klebsiella pneumonia.* Betalactamasas de espectro ampliado (ESBL). Resistencia. Carbapenemasas. BlaIMP-1. BlaCTX-M-15. Líbano.

INTRODUCCIÓN

The patient, a 58 year old man, a heavy smoker with a previous history of gastro-duodenal ulcer, was admitted to the Saint Joseph Hospital-Raymond and Aida Najjar polyclinic (Beirut) on July 17, 2007 when he underwent a surgery for a moderately differentiated colonic adenocarcinoma (T3N0). After surgery, he was given metronidazole 500 mg per day for six days, followed by amoxicillin-clavulanic acid 1.2 g i.v. per day for three days. He was discharged on July 26, 2007, and re-admitted on August 4, 2007 for an abdominal obstruction with septic presentation (leucocytes 28.300/mm^3). He underwent a laparotomy and put on ceftizoxime, a third generation cephalosporin, 1 g/8 hours i.v. until August 16, 2007; in addition, amikacin 500 mg/12 h i.v. was administered for the first three days and followed by gentamicin 80 mg/12 h i.v. for additional three days. Post operatively on day seven, a cutaneous digestive fistula was diagnosed and operated. The wound specimen taken on August 13 grew an extended spectrum beta-lactamase producing *Escherichia coli* susceptible to cefoxitin, imipenem, gentamicin, and nitrofurantoin. Bacteroides vulgatus was as well isolated. The patient was put on imipenem 500 mg per day i.v. and metronidazole 500 mg/8 h i.v. between August 16, 2007 and September 4, 2007. On August 16, the CRP was 173 mg/l and leucocytes count was 7100/mm^3.

On August 28, 2007, a sample was taken from the same sub-cutaneous site where the following bacteria were isolated: *Enterococcus faecium* sensitive to penicillin, ampicillin, gentamicin, rifampicin, and vancomycin, *Pseudomonas aeruginosa* resistant to imipenem, and *Klebsiella pneumoniae resistant* to imipenem (IMP) and showing sensitivity to amikacin.

The patient was discharged on September 4, 2007 without further complications.

The identification of *Klebsiella pneumoniae* strain resistant to imipenem was done using API 20 E for the identification of Enterobacteriaceae (Bio-Merieux, France) and the antimicrobial susceptibility profile was evaluated by the Kirby-Bauer technique. The minimum inhibitory concentrations (MICs) were determined using the Etest method according to Clinical and Laboratory Standards Institute (CLSI) recommendations[1] and the manufacturer's instructions (AB Biodisk, Solna, Sweden). Antimicrobial susceptibility profiles of the *K. pneumoniae* isolate carrying the blaIMP-1 and blaCTX-M genes were as follows: MICs to piperacillin, cefoxitin, ceftriaxone, cefotaxime, ceftazidime, cefpodoxime were >256 µg/ml. MICs to aztreonam, ciprofloxacin, and imipenem were >32 µg/ml, MICs to piperacillin-tazobactam was 64 µg/ml, to ceftazidime-clavulanic acid was >32 and >4.0, and to cefepime was 128 µg/ml. Hydrolysis of IMP was evaluated with bioassays[2] using *S. aureus* ATCC 25923; bioassays involved satellite growth of these strains around the *K. pneumoniae* strain growing on Mueller-Hinton agar plates containing 108 CFU/ml of *S. aureus* ATCC 25923 and IMP at a concentration of 0.06 or 0.12 µg/ml. The production of metallo-β-lactamase (MBL) was detected by a double disk-synergy using ceftazidime and IMP as substrates and ethylene diamine tetraacetic acid (EDTA) and thiol compounds (2-mercaptopropionic acid and 2-mercaptoacetic acid) as β-lactamase inhibitors[3]. The MICs of IMP against the isolated *Klebsiella pneumoniae* with and without EDTA were measured by agar dilution1. To perform the isoelectric focusing, crude β-lactamase extracts in polyacrylamide gels containing ampholines with a pH range of 3.5 to 9.5 were used as previously described[4]. Polymerase chain reaction (PCR) was used for DNA amplification using primers specific to the blaIMP-1 gene and blaCTX-M genes[5,6].

Thiol compounds or EDTA inhibited the imipenemase activity, clavulanate and tazobactam did not. The isolated strain presented an MIC of IMP of 128 µg/ml in the absence of EDTA. The antibacterial activity against *Klebsiella* was restored (1 µg/ml) in the presence of EDTA. This suggests the production of a metallo β-lactamase. The pI of this enzyme was estimated to be >9.5[6]. DNA amplification by PCR yielded a fragment of approximately 600 bp. The phenotype and genotype (DNA amplifications by PCR using primers specific to the blaCTX-M gene yielded a fragment of approximately 550 bp) of the strain strongly suggest that it is producing an ESBL. Isoelectric focusing analysis showed that the isolated strain produced a β-lactamase with a pI of 7.9 which corresponds to a cefotaximase. In view of the wide spread of blaCTX-M-15 in Lebanon, the strain was checked for the presence of this enzyme and the primer used had the following sequence: Rev1: 5'-TGG GTG AAG TAA GTG ACC AGA ATC AGC GG-3', Frw2: 5'-CGA TCC GCG TGA CAC T-3'. The primers were added in a 0.5 µM concentration. The PCR revealed the presence of a 270bp band confirming therefore blaCTX-M-15.

In order to test whether the isolate contained a class 1 integrons, PCR using primers for the 5' and 3' conserved sequences of class 1 integrons was used. The presence of blaIMP-1 and blaCTX-M genes in these integrons was tested by PCR by using a forward primer for the conserved sequence of class 1 integrons with a reverse primer for either blaIMP-1 or blaCTX-M genes and a forward primer for either blaIMP-1 or blaCTX-M genes with a reverse primer for the conserved sequence of class 1 integrons.

DISCUSSION

Beta-lactam antibiotics are widely used in the treatment of bacterial infections. However, the production of extended spectrum beta-lactamases (ESBLs), one of the resistance mechanisms encountered in Enterobacteriaceae, mainly *Escherichia coli* and *Klebsiella* pneumoniae, has been associated with several treatment failures. Indeed, ESBLs are capable of efficiently hydrolyzing extended-spectrum cephalosporins

(cefotaxime and ceftazidime) and are highly susceptible to inhibition by clavulanic acid and tazobactam[7,9,10]. During the last decade, many hospital outbreaks caused by ESBL-producing Enterobacteriaceae spp. have been reported. Most of the ESBL-producing strains carried derivatives of blaTEM-1, blaTEM-2, or blaSHV-1[6,8,11,12]. In a study that constituted the first national surveillance on ESBL-producing Enterobacteriaceae isolated from six Lebanese health care facilities and from the community, it was found that out of 72 nonduplicate ESBL-producing strains that were collected from patients, health care workers, and healthy subjects from different regions of Lebanon, CTX-M-15 was the prevalent ESBL produced. Unlike most CTX-Ms that preferentially hydrolyze cefotaxime, CTX-M-15, an Asp-240-Gly variant of CTX-M-3, increased the catalytic efficiency against ceftazidime[14]. The same Asp-240-Gly substitution has also been reported in CTX-M-16[13]. CTX-M-15 was produced by 83% of the characterized strains and was detected in *E. coli, K. pneumoniae,* and *Enterobacter cloacae* species.

In summary, the data presented here illustrate the complexity and extent of the spread of ESBL-producing Enterobacteriaceae strains in Lebanon. Our results point out mainly the emergence and the dramatic dissemination of CTX-M-15-producing *E. coli* in this country.

Carbapenems, such as IMP, are used more frequently for the treatment of multiresistant gram-negative nosocomial pathogens, especially strains that produce ESBLs. To our knowledge, resistance to these agents among Enterobacteriaceae was not reported yet in our country. Because of the high prevalence of ESBL production in nosocomial strains, selective pressure imposed by the frequent use of carbapenems has led to high levels of resistance to these drugs among strains of gram-negative bacilli in the world[15,16].

Among Enterobacteriaceae, resistance to carbapenems is still rare. In general, this resistance is caused by an overproduction of AmpC enzyme, coupled with alteration in the outer membrane permeability[15,16]. On the other hand, it has been observed more frequently among nonfermentative gram-negative bacilli. Data from Saint George University Hospital in Beirut (Z. Daoud, Personal communication) show that only 61% of the *Acinetobacter baumannii* were susceptible to Imipenem. Currently there have been a growing number of reports indicating an increase in the prevalence of carbapenemases[6,17-19]. Basically, two molecular classes of carbapenem-hydrolyzing enzymes, classes A (Bush group 2f) and B (Bush group 3), have been described. Class B enzymes or MBLs are clinically relevant, since they are able to degrade virtually all β-lactams except monobactams. In contrast to ESBLs, MBLs are not inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam; however, they are inhibited by EDTA and/or thiol compounds[20,21]. Three different types of mobile MBLs have been described in the literature: IMP, VIM, and SPM. IMP and VIM enzymes have been found in various gram-negative clinical isolates, mostly in the Far East and the Mediterranean region[6,16,20]. In Latin America, recent studies have characterized the appearance of metalloenzymes such as IMP and SPM in Brazilian clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa,* respectively[4,17,22].

Among The MBLs detected in *K. pneumoniae,* IMP-1 and IMP-8 have been described in Japan, Singapore, and Taiwan[8,23]. VIM-1 and VIM-4 have recently been described in Greece and Italy[24,25]. Gram-negative bacilli producing both IMP-like and CTX-M enzymes have been recently reported[26]. In our study, we report the presence of both enzymes in the isolated strain from a Lebanese patient, coded by blaIMP-1 and blaCTX-M; similar results were reported by N. Lincopan et al.[27] from Brazil.

Another study by Tato, et al.[28] done in Madrid, Spain showed that the spread of the bla(VIM-1) gene among Enterobacteriaceae was driven by clonal spread associated with intergeneric plasmid transfer with different class I integron platforms. «Such complex epidemiology might anticipate endemicity and should be considered for the design of containment epidemiology strategies» concluded the authors.

To our knowledge, this is the first report of a clinical isolate of *K. pneumoniae* producing an MBL in Lebanon. Although new drugs for the treatment of ESBL producing gram negative bacilli have been introduced to the market; carbapenems are still the main therapeutic option for treating these infections, which are highly prevalent in most Lebanese hospitals[10-12].

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