Advances in Pharmacoepidemiology & Drug Safety

Ou and Lv, Adv Pharmacoepidemiol Drug Saf 2014, 3:2
DOI: 10.4172/2167-1052.1000152

Research Article Open Access

Epidemiological Characteristics of bla_{NDM-1} in *Enterobacteriaceae* and *Acinetobacter calcoaceticus – Acinetobacter baumannii* Complex in China from 2011 to 2012

Weimei Ou and Yuan Lv*

The Institute of Clinical Pharmacology, Peking University First Hospital, China

Abstract

Objectives: The study aimed to investigate the prevalence and the epidemiological characteristics of bla_{NDM-1} in *Enterobacteriaceae* and *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex (ABC) in China from July 2011 to June 2012.

Methods: All organisms studied were screened for the presence of bla_{NDM-1} using PCR. For those bla_{NDM-1}-positive strains, 16S rRNA along with API strips were performed to validate the bacterial genus and species. The ABCs were reconfirmed by PCR detection of $bla_{OXA-51-like}$. The antibiotic susceptibilities were assessed by determining minimum inhibitory concentration (MIC) of them using two-folder agar dilution test recommended by the Clinical and Laboratory Standards Institute (CLSI). Molecular typing was performed using pulsed-field gel electrophoresis (PFGE). An S1 nuclease PFGE (S1-PFGE) and Southern blot hybridization were conducted to ascertain the gene location of bla_{NDM-1}.

Results: Among 2170 the family *Enterobacteriaceae* and 600 ABCs, seven *Enterobacteriaceae* strains and two *A. calcoaceticus* isolates from five different provinces carried the bla_{NDM-1} gene. The seven *Enterobacteriaceae* strains were four *Klebsiella pneumoniae*, one *Enterobacter cloacae*, one *Enterobacter aerogen* and one *Citrobacter freundii*, respectively. All of them showed non-susceptible to any agent of imipenem, meropenem, panipenem and ertapenem. Two *A. calcoaceticus* were both resistant to imipenem and meropenem. Three *K. pneumoniae* showed the same PFGE profiles. Eight bla_{NDM-1} genes were located on plasmids and one on chromosome.

Conclusions: Compared with the previous reports, the numbers and species of the bla_{NDM-1} in *Enterobacteriaceae* have been significantly increased in China and most of them can disseminate which should be drawn great attention. Consecutive surveillance should be implemented and focused on the dissemination of bla_{NDM-1} among gram-negative clinical isolates as well.

Keywords: New Delhi metallo-β-lactamase 1 (NDM-1); *Enterobacteriaceae*; *Acinetobacter baumannii*; Epidemiology

Introduction

Carbapenems are of choice antibiotics to many infections, especially those triggered by milti-drug resistant gram-negative bacteria. Therefore, carbapenemase in clinical gram-negative organisms which can hydrolyze carbapenems are an important threat to public health. What is more wrose, New Delhi metallo-β-lactamase 1(NDM-1), a new type of carbapenemase, can hydrolyze almost all antimicrobials except colistin, tigecycline and sometimes aztreonam, and was thus referred to "superbug" by media. This article will focus on the problem of carbapenem resistance mediated by NDM-1.

NDM-1, a new type of Ambler classer B metallo-β-lactamases (MBLs), encoded by bla_{NDM-1}, was first reported in *K. pneumoniae* and *Escherichia coli* derived from a Swedish patient of Indian origin who was admitted to hospital in New Delhi, India in 2009 [1]. Since then, bla_{NDM-1}-positive bacteria have disseminated worldwide, including almost all seven continents except the Antarctica [2]. Indian subcontinent and China were the major reservoirs, Balkan states like Serbia, Montenegro and Bosnia–Herzegovina may be considered as a 'secondary' reservoir area while the Middle East (Morocco, Algeria, Libya, Egypt, Iraq, Kuwait, Oman, Lebanon and Afghanistan), southeast Asia (South Korea, Indonesia, Vietnam and Thailand) and parts of Europe (France, Italy]) may be additional reservoir areas. The bla_{NDM-1} gene was identified in *K. pneumoniae*, *E. coli*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Proteus spp.*, *Citrobacter freundii*, *Morganella morganii*, *Providencia spp.*, *Acinetobacter spp. and Raoultella ornithinolytica* [3-23]. The bla_{NDM-1} gene was mostly on different large plasmids and partly on chromosome [24]. Those plasmids carrying bla_{NDM-1} were mostly transferable and coexisted with many other resistant determinants [9,11,17], making treatment of NDM-1-producing bacteria a further complication.

This study retrospectively survey the nationwide epidemiology of bla_{NDM-1} in *Enterobacteriaceae* and ABCs strains derived from 18 tertiary hospitals presenting different provinces in China from July 1, 2011 to June 30, 2012.

Materials and Methods

Bacterial strains

The species of the family *Enterobacteriaceae* and ABCs were collected from 18 tertiary hospitals in different provinces in China

***Corresponding author:** Yuan Lv, The Institute of Clinical Pharmacology, Peking University First Hospital, China, Tel: +86-139-1033-2958; E-mail: lyzx5857@163.com

Received March 17, 2014; **Accepted** April 23, 2014; **Published** April 25, 2014

Citation: Ou W, Lv Y (2014) Epidemiological Characteristics of bla_{NDM-1} in *Enterobacteriaceae* and *Acinetobacter calcoaceticus–Acinetobacter baumannii* Complex in China from 2011 to 2012. Adv Pharmacoepidemiol Drug Saf 3: 152. doi:10.4172/2167-1052.1000152

from July 1, 2011 to June 30, 2012. 338 *Enterobacteriaceae* and 395 ABCs which were nonsusceptible to carbepenem were selected from 2170 *Enterobacteriaceae* and 600 ABCs clinical isolates. Standard strains for antimicrobial susceptibility were *E. coli* ATCC25922, *E. coli* ATCC35218 and *Pseudomonas aeruginosa* ATCC27853. *Salmonella* serotype *Braenderup* strain H9812 was used as the marker for PFGE.

PCR amplication

The DNA extraction was performed from fresh culture using boiling techniques. The primers used in this study were based on primers published by the Chinese Center For Disease Control and Prevention (CDC), F:TCG CAT AAA ACG CCT CTG; R:GAA ACT GTC GCA CCT CAT. The reaction mixtures were 20 μl: 2x Tap PCR MIX (TaKaRa, Dalian, China) 10 μl; 20 μM each primer 1 μl; DNA sample 2 μl and ddH_2O 6 μl. Amplification was carried out under the following thermal cycling conditions: 5 min at 94°C; 30 cycles of amplification consisting of 15 s at 94°C, 30 s at 51°C, and 30 s at 72°C; and 10 min at 72°C for the final extension. The amplicon were analyzed by electrophoresis in a 1.5% agarose gel and were sequenced.

Species confirmation

The *bla*$_{NDM-1}$-positive organisms were affirmed for bacterial genus by the sequence analysis of the 16S rRNA, using the universal primers of 27F-AGAGTTTGATCCTGGCTCAG and 1492R-GGCTACCTTGTTACGACTT [25]. The thermal cycling conditions were: 5 min at 94°C; 30 cycles of amplification consisting of 60 s at 95°C, 60 s at 45°C, and 90 s at 72°C; and 10 min at 72°C for the final extension. DNA fragments were visualized by electrophoresis in a 1.5% agarose gel at 110 V for 45 min in 0.5xTBE (45 mM Tris-OH [pH 8.0], 45 mM boric acid, 1mM EDTA) followed by ethidium bromide staining. The PCR products were sequenced and compared with the published sequence in the Genbank database by multiple sequence alignment. To make sure the species, a Kligler Iron Agar assay was carried out first to detect whether the bacteria were ferment or not. The distinction determined using API (bioMe´rieux, Craponne, France) 20E or 20NE to further identify the bacterial species. The non-fermentative bacteria used API 20NE while the rest 20E. The ABCs were distinguished by PCR detection of *bla*$_{OXA-51-like}$ which is intrinsic in *A. baumannii* using primers previously reported as F:TAA TGC TTT GAT CGG CCT TG;R:TGG ATT GCA CTT CAT CTT GG [26].

Antimicrobial susceptibility

Susceptibility testing for *bla*$_{NDM-1}$-positive isolates was performed by determining MICs by two-folder agar dilution test on Mueller-Hinton agar plates at 37°C. The results were interpreted according to the CLSI2013 M100-S23 guidelines [27]. The breakpoints of imipenem and meropenem for family *Enterobacteriaceae* were as follows: susceptible (S), ≤ 1 μg/ml; resistant(R), ≥ 4 μg/ml; for ertapenem were as follows: S, ≤ 0.5 μg/ml; R, ≥ 2 μg/ml. Likewise, the breakpoints of imipenem and meropenem for *A.baumannii* were: S, ≤ 4 μg/ml; R, ≥ 16 μg/ml. Both of the two species, the breakpoints of meropenem were used for panipenem.

PFGE

Bacterial DNA was prepared in agarose blocks and digested with restrict enzyme XbaI (four *K. pneumonia* and *Salmonella* serotype *Braenderup* strain H9812) and ApaI (two *A. calcoaceticus).* The DNA fragments were separated by use of a CHEF-Mapper XA PFGE system (Bio-Rad, USA) at 6 V/cm and 14°C, with a pulse angle of 120°, for 23 h and a switch time from 4 to 40 s in *Enterobacteriaceae* while 24 h and a switch time from 5 to 20s in *A. calcoaceticus.* The gel was stained with ethidium bromide to make the PFGE banding patterns visual.

S1-PFGE and southern hybridization

Refer to the literature published early [28], bacterial DNA was prepared in agarose blocks and digested with S1 nuclease, and then separated by PFGE as above with conditions of 14 h at 6 V/cm and 14°C, with a pulse angle of 120° and a switch time from 1 to 10 s. The gel was stained with ethidium bromide to make the bands visual. After that, the DNA fragments were transferred to nylon membranes (GE, China), hybridized with digoxigenin-labelled *bla*$_{NDM-1}$-specific probes and detected using an NBT/BCIP colour detection kit (Roche, Switzerland).

Plasmid analysis and southern hybridization

Plasmids were extracted according to Molecular Cloning: a laboratory manual then digested with *EcoR I* and agarose gel electrophoresis at 90v 45 min after prepared in agarose holes. The gel was stained with ethidium bromide to make the plasmid profiles visual. The plasmid fragments were then transferred to nylon membranes hybridized with digoxigenin-labelled *bla*$_{NDM-1}$-specific probes and detected using an NBT/BCIP colour detection kit as above.

Results

The identification of *bla*$_{NDM-1}$-positive bacteria

All PCR detection for *bla*$_{NDM-1}$ results was positive. The sequencing

Strains	PRL	TZP	CTX	CRO	CAZ	CFP	SCF	FEP	ATM	IMP	MEM	PAN	ETP	GEN	AMK	TCY	MNO	TGC	CIP	LVP	NIT	POL	POS
M186	512	512	256	512	512	512	256	32	512	4	8	16	32	0.5	1	0.5	1	0.5	0.031	0.031	128	—	2
M187	512	512	256	512	512	512	512	64	512	4	8	16	16	0.5	1	1	1	0.5	0.031	0.031	128	—	4
M194	512	512	256	512	512	512	512	64	512	8	8	32	16	0.5	1	2	1	0.5	0.031	0.062	128	—	4
U091	512	256	256	256	512	512	256	32	64	4	8	8	8	1	0.25	256	64	0.5	0.25	0.5	32	—	8
Q297	512	256	512	256	512	512	512	64	256	4	8	64	32	128	2	128	64	1	0.5	2	64	—	2
Q442	512	512	512	512	512	512	512	64	256	2	4	8	16	0.25	1	256	128	4	4	4	128	—	4
X122	256	256	256	256	512	256	256	32	64	2	2	8	8	32	0.5	128	16	0.5	8	8	16	—	0.3
G113	256	256	>256	>256	>256	—	128	>256	—	128	128	—	—	256	8	2	0.125	—	0.25	0.5	—	0.5	—
X231	256	256	512	512	512	512	256	>256	—	128	128	256	—	1	2	2	0.062	0.1	0.062	0.062	—	1	128

PRL:Piperacillin. TZP: Piperacillin/tazobactam. CTX: Cefotaxime. CRO: Ceftriaxone. CAZ: Ceftriaxone. CFP: Cefoperazone. SCF: Cefoperazone/sulbactam2:1. FEP: Cefepime. ATM: Aztreonam. IMP: Imipenem. MEM: Meropenem. PAN: panipenem. ETP: Ertapenem. GEN: Gentamicin. AMK: Amikacin. TCY: Tetracycline. MNO: Minocycline. TGC:Tigecycline. CIP: Ciprofloxacin. LVP: Levofloxacin. NIT: Nitrofurantoin, POL: Polymyxin, B. POS: Phosphonomycin. USA-FDA breakpoint was applied for tigecycline (S: ≤ 2 mg·L^{-1}; R: ≥ 8 mg·L^{-1}) in both *Enterobacteriaceae* and *A.baumannii.* —:None tested.

Table 1: The MICs of bla$_{NDM-1}$-positive bacteria.

results of the amplicons showed all were 100% identity with *K. pneumoniae* strain 05-506 (Genbank accession number: FN396876). 16S rRNA sequencing and biochemical API strips revealed that four were *K. pneumoniae* (M186, M187, M194, U091), two were ABCs (G113, X231), one was *Enterobacter cloacae* (Q297), one *Enterobacter aerogenes* (Q442) and one *Citrobacter freundii* (X122), respectively. The $bla_{OXA-51-like}$ detection of the two ABCs was negative, indicating that both G113 and X231 were *A. calcoaceticus.*

Adv Pharmacoepidemiol Drug Saf, an open access journal
ISSN: 2167-1052

The MICs of bla_{NDM-1}-positive bacteria

All nine strains showed highly resistant to broad spectrum penicillin, cephalosporins, β-lactamase inhibitor combinations, most carbapenem and nitrofurantoin, but showed variable susceptibilities to aminoglycosides and tetracyclines. The good news was that most strains show susceptible to fluoroquinolones and tigycycline (Table 1).

PFGE

The three *K. pneumonia* from the same provinces (M186, M187, M194) had the same PFGE profiles while the two *A. calcoaceticus* showed different profiles (Figure 1).

Plasmid analysis of bla_{NDM-1}-positive bacteria

Except U091 on chromosome and X122 not succeed, the other seven strains all displayed the bla_{NDM-1} gene were on plasmid, with size ranging from ~23 to ~96 kb (Figure 2).

Plasmid analysis and southern hybridization

Since S1-PFGE results weren't good and repeated results weren't stable, we conducted plasmid extraction and Southern blot to make sure whether the bla_{NDM-1} was on plasmid or not. U091 didn't have plasmid, which could further explain the above S1-PFGE & Southern blot result

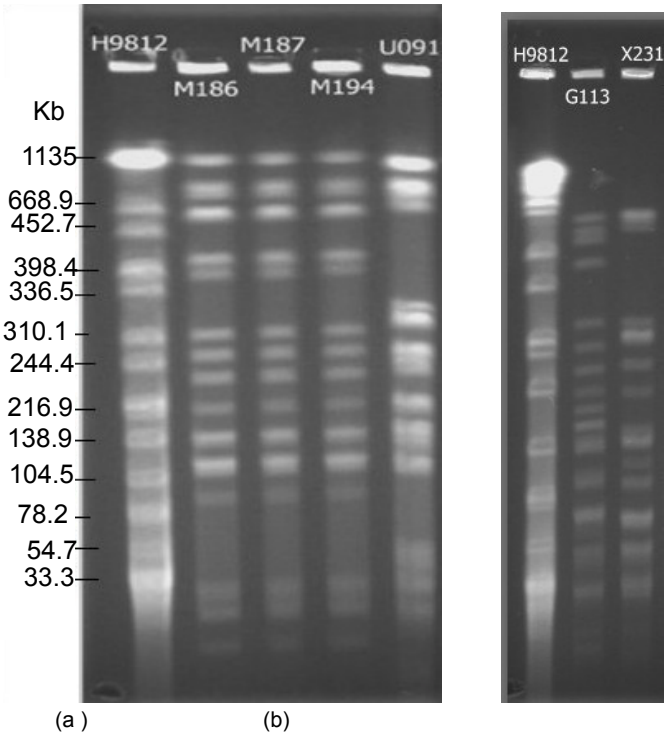

Figure 1: PFGE profiles of four *K.pneumonia*(a) and two *A. calcoaceticus*(b).

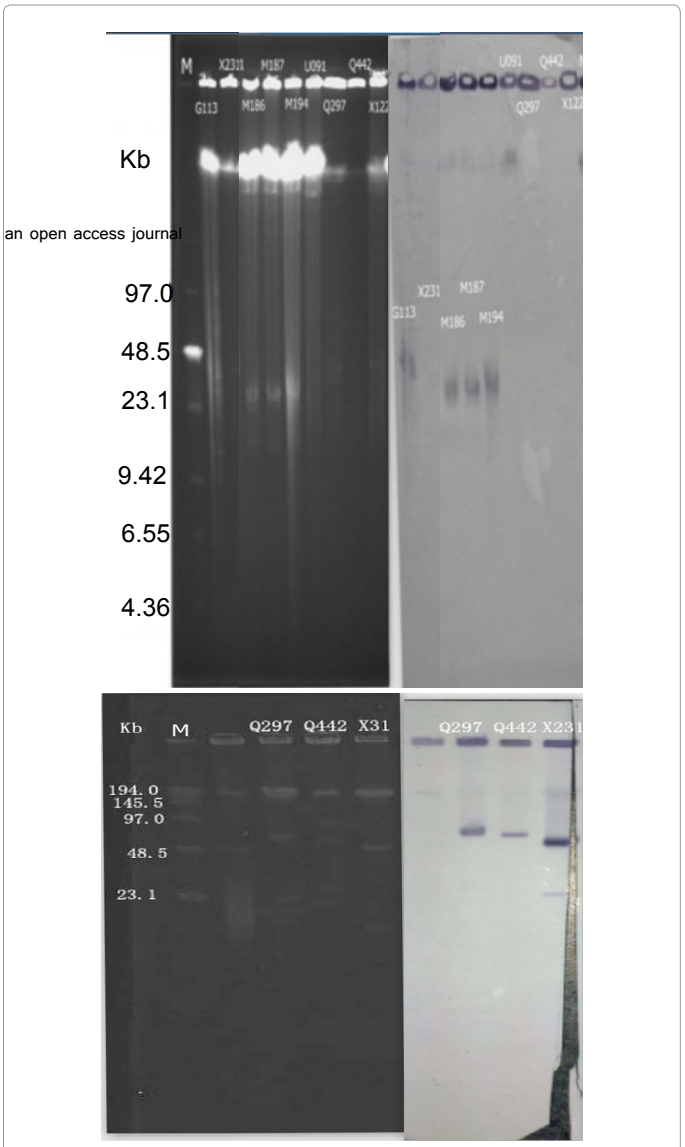

Figure 2: Results of S1-PFGE(left) and Southern blot hybridization(right).M:Low Range PFG Marker.

that why its bla_{NDM-1} gene was on chromosome. Other eight strains all had plasmids. The sizes of plasmids were consistent with S1-PFGE results above. Southern blot hybridation showed the bla_{NDM-1} gene were all on plasmids in the eight strains that haboured plasmids (Figure 3).

Discussion

In the past few decades, an alarming increase in the prevalence of antimicrobial resistant pathogens of serious community- and hospital-acquired infections has been shown worldwide. The increase in carbapenem resistance in Gram-negative bacteria has become a major concern. Bacteria producing NDM-1 had ever caused global panic because they can hydrolyze almost all antimicrobial agents except few, which were referred to "Superbug" by media. To further complicate matters, the bla_{NDM-1} gene encoding NDM-1 has disseminated rapidly over distantly related geographical areas around the world [8,12,18,23]. In terms of human hosts, there are three major routes to acquire an NDM-1 producing organism: nosocomial, personal travel and

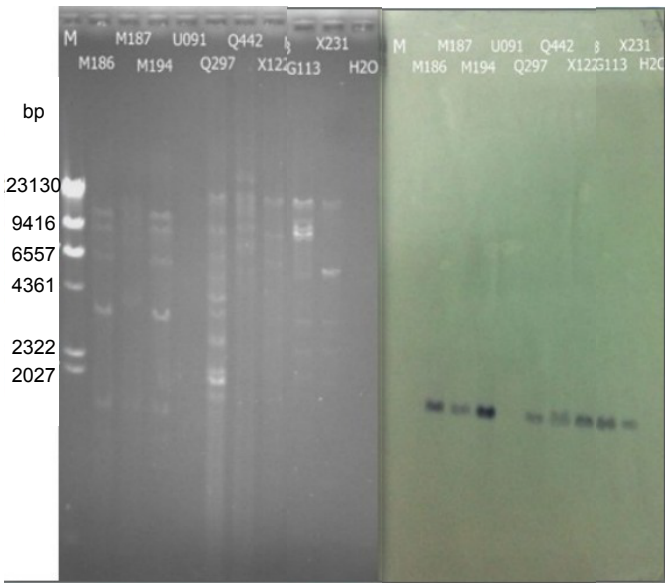

Figure 3: Plasmid profiles and Southern blot results.M:λDNA\HindIII.

community acquisition. The bla_{NDM-1}-carrying bacteria have been reported as gut colonizers in human with or without clinical symptoms, they can survive in the local environment as well, which may result in human acquiring the bla_{NDM-1}-positive bacteria unconciously. Hence, the bacteria possessing the bla_{NDM-1} gene constitute tremendous health threat to human.

There are three main resistant mechanisms against β-lactam antibiotic: 1.the production of β-lactamases which cleave the amide bond of the β-lactam ring; 2.the possession of an altered or acquired penicillin binding protein with low affinity for β-lactams; 3.over expression of efflux pump mechanism [29]. The most common mechanism of resistance in carbapenem is the production of carbapenemases (one of β-lactamases), including enzymes of Ambler classes A, D and B (MBLs). NDM-1 is one of MBLs that mediates carbapenem-resistant. In this study, we confirmed those bla_{NDM-1}-bearing strains were resistant if not intermediate to carbapenem.

China has reported bla_{NDM-1}-producing bacteria since 2010 by Chinese CDC. Since then, many researches have been on this issue. Chen et al. [13] reported four *A. baumannii* at the mainland of China, Ho et al. [17] reported one *E.coli* in Hong Kong, Wu et al. [20] reported *K. pneumoniae* in Tai Wan. By now, a number of bla_{NDM-1}-positive bacteria have been reported. So far, the species China has discovered have *E. coli, K. pneumoniae, K. oxytoca, K. ozaenae, E. cloacae, E. aerogen, C. freundii, Salmonella enteritidis, Morganella morganii, Providencia spp., Alcaligenes faecalis, Kocuria varians, Moraxella group, Comamonas testosteroni, Stenotrophomonas maltophilia, Staphylococcus capitis, Methylobacterium species, Raoultella ornithinolytica, Acinetobacter spp.* and *E. faecium*. Though there are several molecular level researches on the genetic context of bla_{NDM-1}, there are limited studies about epidemiology of bla_{NDM-1}-containing isolates [6,10,13-15,17]. Compared with previously reports [10,13], this study demonstrates that the numbers and species of bla_{NDM-1} in family *Enterobacteriaceae* have been significantly increased in China ,including *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterobacter aerogen* and *Citrobacter freundii*. This situation should draw intensive attention since *Enterobacteriaceae* are the main cause of nosocomil infection. It will be undoubtedly much troublesome once they acquire $bla_{NDM-1.}$

Our study reported nine bla_{NDM-1}-producing strains in all. Except the U091 strain the bla_{NDM-1} gene was on chromosome, all other eight Southern blot hybridization results showed that bla_{NDM-1} were all on plasmid, which may result in horizontal transmission rapidly. Further studies are being done to elucidate the transmissibility and the background of resistance determinants.

References

1. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, et al. (2009) Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother 53: 5046-5054.
2. Wailan AM, Paterson DL (2014) The spread and acquisition of NDM-1: a multifactorial problem. Expert Rev Anti Infect Ther 12: 91-115.
3. Wang SJ, Chiu SH, Lin YC, Tsai YC, Mu JJ (2013) Carbapenem resistant *Enterobacteriaceae* carrying New Delhi metallo-beta-lactamase gene (NDM-1) in Taiwan. Diagn Microbiol Infect Dis 76: 248-249.
4. Zhou G, Guo S, Luo Y, Ye L, Song Y, et al. (2014) NDM-1-producing strains, family *Enterobacteriaceae*, in hospital, Beijing, China. Emerg Infect Dis 20: 340-342.
5. Du XX, Wang JF, Fu Y, Zhao F, Chen Y, et al. (2013) Genetic characteristics of blaNDM-1-positive plasmid in *Citrobacter freundii* isolate separated from a clinical infectious patient. J Med Microbiol 62: 1332-1337.
6. Zhou WQ, Zhang ZF, Shen H, Ning MZ, Xu XJ, et al. (2013) First report of the emergence of New Delhi metallo-beta-lactamase-1 producing *Acinetobacter junii* in Nanjing, China. Indian J Med Microbiol 31: 206-207.
7. Decousser JW, Jansen C, Nordmann P, Emirian A, Bonnin RA, et al. (2013) Outbreak of NDM-1-producing *Acinetobacter baumannii* in France, January to May 2013. Euro Surveill 18.
8. Bonnin RA, Poirel L, Naas T, Pirs M, Seme K, et al. (2012) Dissemination of New Delhi metallo-Î²-lactamase-1-producing Acinetobacter baumannii in Europe. Clin Microbiol Infect 18: E362-365.
9. Karthikeyan K, Thirunarayan MA, Krishnan P (2010) Coexistence of blaOXA-23 with blaNDM-1 and armA in clinical isolates of *Acinetobacter baumannii* from India. J Antimicrob Chemother 65: 2253-2254.
10. Ho PL, Li Z, Lai EL, Chiu SS, Cheng VC (2012) Emergence of NDM-1-producing *Enterobacteriaceae* in China. J Antimicrob Chemother 67: 1553-1555.
11. Chen Z, Qlu S, Wang Y, Wang Y, Liu S, et al. (2011) Coexistence of blaNDM-1 with the prevalent blaOXA23 and blaIMP in pan-drug resistant *Acinetobacter baumannii* isolates in China. Clin Infect Dis 52: 692-693.
12. Centers for Disease Control and Prevention (CDC) (2010) Detection of *Enterobacteriaceae* isolates carrying metallo-beta-lactamase - United States, 2010. MMWR Morb Mortal Wkly Rep 59: 750.
13. Chen Y, Zhou Z, Jiang Y, Yu Y (2011) Emergence of NDM-1-producing *Acinetobacter baumannii* in China. J Antimicrob Chemother 66: 1255-1259.
14. Fu Y, Du X, Ji J, Chen Y, Jiang Y, et al. (2012) Epidemiological characteristics and genetic structure of blaNDM-1 in non-baumannii *Acinetobacter spp.* in China. J Antimicrob Chemother 67: 2114-2122.
15. Liu Z, Li W, Wang J, Pan J, Sun S, et al. (2013) Identification and characterization of the first *Escherichia* coli strain carrying NDM-1 gene in China. PLoS One 8: e66666.
16. Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Göttig S, et al. (2011) Molecular characterization of blaNDM-1 in an *Acinetobacter baumannii* strain isolated in Germany in 2007. J Antimicrob Chemother 66: 1998-2001.
17. Ho PL, Lo WU, Yeung MK, Lin CH, Chow KH, et al. (2011) Complete sequencing of pNDM-HK encoding NDM-1 carbapenemase from a multidrug-resistant *Escherichia coli* strain isolated in Hong Kong. PLoS One 6: e17989.
18. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, et al. (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10: 597-602.
19. Solé M, Pitart C, Roca I, Fàbrega A, Salvador P, et al. (2011) First description of an *Escherichia coli* strain producing NDM-1 carbapenemase in Spain. Antimicrob Agents Chemother 55: 4402-4404.

20. Wu HS, Chen TL, Chen IC, Huang MS, Wang FD, et al. (2010) First identification of a patient colonized with *Klebsiella pneumoniae* carrying blaNDM-1 in Taiwan. J Chin Med Assoc 73: 596-598.

21. Poirel L, Hombrouck-Alet C, Freneaux C, Bernabeu S, Nordmann P (2010) Global spread of New Delhi metallo-beta-lactamase 1. Lancet Infect Dis 10: 832.

22. Wang Y, Wu C, Zhang Q, Qi J, Liu H, et al. (2012) Identification of New Delhi metallo-beta-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. PLoS One 7: e37152.

23. Boulanger A, Naas T, Fortineau N, Figueiredo S, Nordmann P (2012) NDM-1-producing *Acinetobacter baumannii* from Algeria. Antimicrob Agents Chemother 56: 2214-2215.

24. Poirel L, Dortet L, Bernabeu S, Nordmann P (2011) Genetic features of blaNDM-1-positive *Enterobacteriaceae*. Antimicrob Agents Chemother 55: 5403-5407.

25. Kim TW, Kim YH, Kim SE, Lee JH, Park CS, et al. (2010) Identification and distribution of *Bacillus* species in doenjang by whole-cell protein patterns and 16S rRNA gene sequence analysis. J Microbiol Biotechnol 20: 1210-1214.

26. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, et al. (2006) Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter spp*. Int J Antimicrob Agents 27: 351-353.

27. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Tweny-Third Informational Supplement M100-S23. CLSI, Wayne, PA, USA, 2013.

28. Barton BM, Harding GP, Zuccarelli AJ (1995) A general method for detecting and sizing large plasmids. Anal Biochem 226: 235-240.

29. Poirel L, Walsh TR, Cuvillier V, Nordmann P (2011) Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70: 119-123.