

## RESEARCH NOTE

# DETECTION OF NEW DELHI METALLO-BETA-LACTAMASE-1-PRODUCING *KLEBSIELLA PNEUMONIAE* AT A GENERAL HOSPITAL IN THAILAND

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**Abstract.** The purpose of this study was to detect carbapenemase genes in clinical isolates of carbapenem-resistant Enterobacteriaceae (CRE) obtained from patients admitted to Hua-Hin Hospital, Prachuab Khiri Khan Province, Thailand between January and December 2014. Screening of CRE was initially determined using disk diffusion method, and subsequently using modified Hodge test (MHT). Multiplex PCR was employed to amplify carbapenemase genes, *bla*<sub>IMP</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>NDM</sub>, *bla*<sub>KPC</sub> and *bla*<sub>VIM</sub>. Of the 624 clinical isolates, seven CRE isolates were identified by the disk diffusion method, but were negative for MHT. Only one isolate, *Klebsiella pneumoniae*, was found to carry *bla*<sub>NDM-1</sub> encoding New Delhi metallo-β-lactamase-1, and the remaining CRE isolates were negative for the carbapenemase genes looked at. However, monitoring of carbapenem resistance among Enterobacteriaceae should be for optimal infection control measures.

**Keywords:** Enterobacteriaceae, *Klebsiella pneumoniae*, carbapenemase, general hospital, New Delhi metallo-β-lactamase-1

### INTRODUCTION

Enterobacteriaceae, gram-negative rod-shaped bacteria, play a major role as causative human pathogens in various organs, such as the urinary tract, lower respiratory tract, blood, and abdomen in patients in community and healthcare setting (Tangden and Giske, 2015). Over

the past fourteen years, there has been an increase in the prevalence of extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella pneumoniae* (Livermore, 2012). For instance, Sader *et al* (2014) reported that among *Klebsiella* spp isolated from hospitalized bacteremic patients with urinary tract infections in the USA and European Union the rate of ESBL-producing bacteria increases from 11.4% and 17.1% in 2009 to 16.1% and 40.4% in 2011, respectively. The Study for Monitoring Antimicrobial Resistance Trends (SMART), conducted to investigate gram-

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negative bacteria from patients with intra-abdominal infections in the Asia Pacific region between 2002 - 2006, reported a decrease in susceptibility rates of Enterobacteriaceae isolates to cephalosporins and an increase in the prevalence of ESBL-producers from 13% in 2002 to 28% in 2006 (Ko and Hsueh, 2009). A study conducted under The National Antimicrobial Resistance Surveillance Thailand (NARST) program that involved more than 30 hospitals throughout the country during 2000-2005 revealed an increase in the incidence rate of ESBL-producing *E. coli* (Apisarntharak *et al*, 2009). Recently, Hongsuwan *et al* (2014) reported that among 10 provincial hospitals in northeast Thailand there is an overall increase during 2004 to 2010 in the proportions of ESBL-producing *E. coli* that cause hospital-acquired bacteremia (from 33.3% to 51.5%) and healthcare-associated bacteremia (from 20.8% to 32.9%).

The increase in ESBL-producing pathogens has affected treatment of Enterobacteriaceae infections with third- and fourth-generation cephalosporins. However, carbapenems, beta lactams/ beta lactamase inhibitors, and non-beta lactams antimicrobials, such as aminoglycosides, fosfomicin, and a number of fluoroquinolones are still applicable (Curello and MacDougall, 2014). Consequently, carbapenems have been widely used for treating infections from ESBL-producing bacteria. Unfortunately, Enterobacteriaceae will continue to adapt under antibiotic pressure, and carbapenem-resistant Enterobacteriaceae (CRE) has been reported recently (Tangden and Giske, 2015).

To date, carbapenem-destroying enzymes have been found in several types of classes, namely, A, B, and D of the Amber system, which include IMP, VIM, OXA-48, New Delhi metallo- $\beta$ -lactamase (NDM), and *K. pneumoniae* carbapenemase (KPC),

originally isolated in 1996 in the United States (Nordmann, 2014). Since then, KPCs have spread throughout various parts of the world and are found mostly in clinical isolates (Nordmann, 2014; Tangden and Giske, 2015).

In Thailand, there has been a report of NDM-1 and IMP-14 carbapenamases from the northeast region (Rimrang *et al*, 2012) and one of KPC-13-producing Enterobacteriaceae from the central part (Netikul *et al*, 2014a). In Hua-Hin General Hospital, Prachuab Khiri Khan Province, located to the south of Bangkok, a number of clinical isolates resistant to carbapenems have been found during June-December 2013 (Preechachuawong P, Hua-Hin Hospital, personal communication). However, the carbapenem resistance phenotype did not represent known pathogens harboring carbapenemase genes. This study sought to identify carbapenemase genes present in these carbapenem-resistant Enterobacteriaceae clinical isolates.

## MATERIALS AND METHODS

### Bacterial strains

All clinical Enterobacteriaceae isolates were obtained from patients admitted to the 400-bed Hua-Hin General Hospital between January and December 2014. Carbapenem-phenotypic-resistant Enterobacteriaceae was defined as isolates that were intermediate or resistant to ertapenem (10  $\mu$ g), imipenem (10  $\mu$ g), or meropenem (10  $\mu$ g), and usually were resistant to one or more third-generation cephalosporin [eg, cefotaxime (30  $\mu$ g), ceftazidime (30  $\mu$ g), and ceftriaxone (30  $\mu$ g)] using the disk diffusion method (CLSI, 2014). All CRE clinical isolates from various specimens were kept in tryptic soy broth containing 20% glycerol at -80°C until used. The research protocols

were approved by the Ethics Committee with a waiver for informed consent (No. ID004/2558).

#### Phenotypic detection of carbapenemases

The modified Hodge test (MHT) based on CLSI criteria was used as phenotypic test for the presence of carbapenemases (CLSI, 2014). In brief, 0.5 McFarland turbidity of *E. coli* ATCC 25922 in normal saline solution (NSS) was diluted ten-fold in NSS and inoculated in a Mueller-Hinton agar plate for antimicrobial disk diffusion testing. Ertapenem (10 µg)- or meropenem (10 µg)-containing disk was placed on the dry inoculated plate. Three to five colonies of test or quality control strains (MHT-positive *K. pneumoniae* ATCC BAA-1705 and MHT-negative *K. pneumoniae* ATCC BAA-1706) were grown overnight, and inoculated in a straight line (20-25 mm in length) from the edge of the meropenem disk. After 16-24 hours of incubation, MHT-positive result shows a clover leaf-like indentation of the *E. coli* 25922 that grew along the test organism within the disk diffusion zone, and indicates that the test microorganism produces carbapenemase(s).

#### PCR amplification of carbapenemase genes

DNA of clinical strains was isolated using a commercial DNA extraction kit (RBC Bioscience, New Taipei City, Taiwan). The 30-µl PCR mixture consisted of 2 µl of DNA, 0.8 µl of 20 µM each forward and reverse primers (listed in Table 1), 15 µl of PCR master mix kit (JumpStart Red Taq® Ready Mix; Sigma, St Louis, MO) and 3 µl of DNAase-free water. Thermocycling (conducted in Biometra PCR instrument; Biometra, Göttingen, Germany) conditions were as follows: 94°C for 3 minutes; 35 cycles of 94°C for 30 seconds, 56°C for 35 seconds, and 72°C for 45 seconds; and a final step at 72°C for 5 minutes. Ampli-

cons were separated by 2.0% agarose gel electrophoresis, stained with ethidium bromide, and detected with a UV transilluminator equipped with a camera. The amplicon sizes were compared with those of known carbapenemase genes and their identities were confirmed by direct nucleotide sequencing (Ward Medic, Bangkok, Thailand) and comparison with GenBank database.

## RESULTS

During the study period, from a total of 624 clinical isolates, there were seven (1.1%) non-susceptible carbapenem Enterobacteriaceae isolates, three of which were *K. pneumoniae*, *Enterobacter* sp and *Proteus* sp. The seven samples were isolated from urine ( $n = 2$ ), pus ( $n = 2$ ), sputum ( $n = 1$ ), blood ( $n = 1$ ), and peritoneal dialysate fluid ( $n = 1$ ) specimens. None of the isolates were positive on the modified Hodge test (Table 2).

Sequencing of PCR amplicons obtained using carbapenemase gene-specific primers (Table 1), revealed only a single *K. pneumoniae* isolate (from urine) carrying *bla*<sub>NDM</sub>, a prevalence of 0.3% (Fig 1).

## DISCUSSION

Carbapenemase-producing Enterobacteriaceae have increased dramatically in various parts of the world (Tangden and Giske, 2015). At least three groups based on the Amber classification have been reported, namely, class A beta-lactamase (KPC), class B (metallo-enzymes: IMP, VIM, NDM), and class D (OXA-48 type (Nordmann, 2014). The present study is the third report of NDM-type carbapenemase genes (Rimrang *et al*, 2012; Netikul *et al*, 2014b) in Thailand but is the first report of *bla*<sub>NDM</sub>-containing Enterobacte-

Table 1  
Primers used to identify carbapenemase genes.

Gene	Primer sequence <sup>a</sup>	Amplicon size (bp)
IMP	F- 5'-GGAATAGAGTGGCTTAAYTCTC-3' R- 5'-GGTTTAAAYAAAACAACCA C-3'	232
VIM	F- 5'-GATGGTGTGTTGGTCGCATA-3' F- 5'-CGAATGCGCAGCACCAG-3'	390
OXA-48	F- 5'-GCGTGGTTAAGGATGAACAC-3' R- 5'-CATCAAGTTCAACCCAACCG-3'	438
NDM	F- 5'-GGTTTGGCGATCTGGTTTTTC-3' R- 5'-CGGAATGGCTCATCACGATC-3'	621
KPC	F- 5'-CGTCTAGTTCTGCTGTCTTG-3' R- 5'-CTTGTCATCCTTGTTAGGCG-3'	798

<sup>a</sup>From Poirel *et al* (2011). Y = (pYrimidine; C or T).

Table 2  
Characteristics of the seven non-susceptible carbapenem Enterobacteriaceae clinical isolates.

Pathogen	Specimen	Carbapenem sensitivity <sup>a</sup>			Modified Hodge test
		Ertapenem (10 µg)	Meropenem (10 µg)	Imipenem (10 µg)	
<i>Proteus</i> spp	Pus	S	S	I	Negative
<i>Proteus</i> spp	Urine	S	S	I	Negative
<i>K. pneumoniae</i>	Urine	R	R	R	Negative
<i>Enterobacter</i> spp	Peritoneal dialysate fluid	R	S	I	Negative
<i>Proteus</i> spp	Sputum	S	S	R	Negative
<i>Proteus</i> spp	Pus	S	S	I	Negative
<i>Enterobacter</i> spp	Blood	I	S	S	Negative

I, intermediate; R, resistant; S, sensitive. <sup>a</sup>Based on disk diffusion method.

riaceae in a general hospital rather than a medical school hospital. Similarly, Wang *et al* (2015) found that CRE strains could be isolated from patients at 2 major medical centers (one of them being a university hospital located in northern Taiwan) and 2 regional hospitals located in central and southern Taiwan, respectively. Thus, although the prevalence of CRE in Thailand

is still low, careful monitoring in different types of hospital is urgently needed.

NDM-type carbapenemase in Amber class B is one of the most commonly reported, being first identified in a Swedish patient who had returned from New Delhi (Nordmann, 2014). The main reservoirs of this resistant strain are found on the Indian sub-continent (Bangladesh, India,

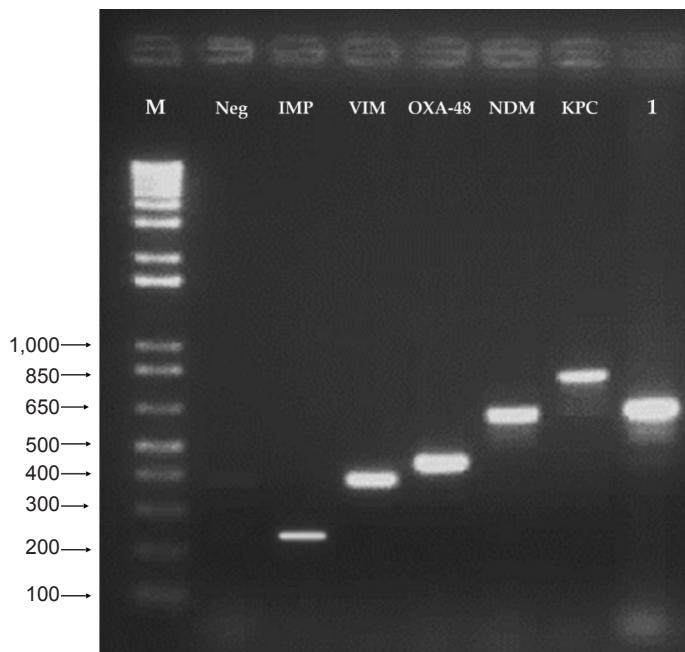


Fig 1—PCR detection of presence of NDM-1 gene in a *Klebsiella pneumoniae* isolate. PCR amplification protocol is described in Materials and Methods. M, molecular size markers [size (bp) is indicated in the left margin]; Neg, negative control; IMP, positive control; VIM, positive control; OXA-48, positive control; NDM, positive control; KPC, positive control; 1, test sample.

Pakistan, and Sri Lanka), but to date, *bla*<sub>NDM</sub>-containing Enterobacteriaceae have been found in Australia, Canada, France, Great Britain, Kenya, Malaysia, Saudi Arabia, South Africa, and USA (Nordmann, 2014). We can only speculate as to the origin of this *bla*<sub>NDM</sub>-carrying *K. pneumoniae* strain, but Hua-Hin is a popular destination for foreign tourists. Previous studies have shown that this strain can be spread via infected patients and/or by air transportation (Dortet *et al*, 2008; Jain *et al*, 2014).

Not surprisingly, the NDM-1 type isolate was not positive on the modified Hodge test. A previous study indicated

that the modified Hodge test performs poorly in the detection of metallo-β-lactamase-producing Enterobacteriaceae isolates (Doyle *et al*, 2012).

No other carbapenemase genes (*bla*<sub>OXA-48</sub>, *bla*<sub>KPC</sub>, *bla*<sub>IMP</sub>, or *bla*<sub>VIM</sub>) were detected in the present study. This might be due to the small sample size, limited period of sample collection, or their extremely low prevalence in the hospital setting as a previous report could not detect any MBL in *Acinetobacter baumannii* other than OXA-23 and OXA-40 types (Santimaleeworagun *et al*, 2014). Nevertheless, identification of carbapenemase-producing Enterobacteriaceae should be evaluated in order to prevent their spread among other in-patients and to the community at large.

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