First Report of NDM-1-Producing Acinetobacter guillouiae

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FIRST REPORT OF NDM-1 PRODUCING *ACINETOBACTER GUILLONIAE*

ABSTRACT

Since 2014 screening for metallo-β-lactamases (MBLs) of all *Acinetobacter* spp isolates by phenotypic methods and PCR has been implemented at the University Hospital Center Zagreb. The first MBL positive isolate was identified in *Acinetobacter guiolloniae*. The strain was isolated from drain of a newborn child hospitalized in a paediatric intensive care unit for heart malformation and identified as *Acinetobacter guiolloniae* with Maldi Tof automated system. The child was teated with meropenem and vancomycin prior to isolation of this strain. The strain was resistant to meropenem, imipenem, ceftazidime, cefotaxime, ceftriaxone, cefepime, gentamicin and ciprofloxacin and sulbactam/ampicillin, intermediate susceptible to piperacillin/tazobactam, and susceptible to colistin. Hodge test and combined disk test with EDTA were positive indicating the production of MBL. PCR and sequencing revealed $bla_{\text{OXA-58}}$ and $bla_{\text{NDM-1}}$ genes. This is the first report of NDM-1 in *Acinetobacter* spp in Croatia. Early detection of these genes will help in prevention and adequate infection control by limiting the spread of these organisms.

Key words: NDM-1, carbapenemases, OXA-58, *Acinetobacter guiolloniae*

To the editor: *Acinetobacter spp* is an opportunistic pathogen with increasing relevance in nosocomial infections (1-2). It is often associated with pneumonia, septicemia, urinary tract infections, wound infections and meningitis. Carbapenem-resistant strains have been reported all over the world. The first outbreak of carbapenem-resistant *Acinetobacter baumannii* isolates in Croatia has been documented in Split University Hospital (3). As demonstrated later, the carbapenem-resistance was mediated by hyperproduction of OXA-51 due the IS*Aba1* location upstream of the genes (4). Later, the studies of carbapenem-resistance in Croatia have reported the emergence of OXA-23, OXA-58 and OXA-72 producing strains in different hospital centers in Croatia (5-7) but no metallo-β-lactamases (MBL) were reported. Since 2014 screening for (MBLs) of all *Acinetobacter* spp isolates by phenotypic methods and PCR has been implemented at the University Hospital Center Zagreb. The first MBL was identified in *Acinetobacter guiolloniae*. The strain was isolated from drain of a newborn child hospitalized in a paediatric intensive care unit for heart malformation and identified as *A. guiolloniae* with Maldi Tof automated system. The child was teated with meropenem and vancomycin prior to isolation of this strain.

Antimicrobial drug susceptibility testing was performed by Vitex2 (bioMeriux, Marcy-l'Etoile, France) and broth micodilution test and interpreted according to CLSI (8). The strain was resistant to meropenem (MIC= 32 µg/ml), ceftazidime, cefotaxime, ceftriaxone, cefepim and sulbactam/ampicillin with MIC of >128 µg/ml, gentamicin (MIC=8 mg/L) and ciprofloxacin (MIC=4 µg/ml), intermediate susceptible to piperacillin/tazobactam (MIC= 64 µg/mL) and imipenem (8 µg/ml), and susceptible to amikacin (MIC=2 µg/ml) and colistin (MIC=0.25 µg/ml). Hodge test and combined disk test with EDTA were positive indicating the production of MBL. MICs of meropenem and imipenem were not reduced by cloxacillin indicating that chromosomal AmpC β-lactamase did not affect susceptibility to carbapenems, but small reduction of two dilutions was observed after addition of sodium chloride

indicating the production of OXA-58. Combined disk test with clavulanic acid was negative which is consistent with the absence of ESBL. PCR and sequencing of chromosomal DNA from boiled colonies revealed $bla_{\text{OXA-58}}$ and $bla_{\text{NDM-1}}$ genes. Plasmid was extracted with Qiagen Mini kit (Inel, Zagreb, Croatia) and subjected to PCR with primers specific for $bla_{\text{OXA-58}}$ and $bla_{\text{NDM-1}}$ gene but yielded no product indicating chromosomal origin of the genes. Incompatibility group of the plasmids described in *A. baumannii* was determined by multiplex PCR with six primer pairs covering 19 homology groups according to Bertini et al. (9). Plasmid extraction did not yield any products in mulitiplex PCR for incompatibility groups described so far. Class 1 integron was amplified with primers CSU-F and CSU-R as described previously (11). $Bla_{\text{NDM-1}}$ gene was encoded in class 1 integron. This is the first report of NDM-1 in *Acinetobacter* spp in Croatia. Previous studies reported NDM-1 in *A. baumannii* in Czech republic in a patient repatriated from Egypt (11), in Lebanon from civilians wounded during the Syrian war (12), in East Africa (13), Iran (14) and Belgium (15). Outbreaks associated with NDM-1 positive *A. baumannii* were described in France (16). In India, coexistence of OXA-23 and NDM-1 was reported (17). In our study coproduction of NDM-1 and OXA-58 β-lactamase was described. The presence of IS*Aba*3 upstream of $bla_{\text{OXA-58}}$ gene promotes the expression of the gene and the level of carbapenem resistance and plays role in the mobilization of the gene. NDM-1 is hydrolyzing virtually all β-lactams but the strain was intermediate susceptible to piperacillin/tazobactam and imipenem. The level of β-lactam resistance in MBL positive strains depends on the expression of bla_{MBL} gene and it is possible that A. *guiolloniae* has low level expression of the gene due to low gene copy number or weak promotor which results in small amount of enzyme produced. OXA-58 and NDM-1 β-lactamases are usually plasmid-mediated (7, 19) but our strain yielded no amplicon with plasmid extract indicating chromosomal origin of the β-lactamases. $Bla_{\text{NDM-1}}$ gene did not spread to other more pathogenic species in the genus *Acinetobacter.*

Due to effective infection control measures the strain did not spread to other patients or other hospital units. It was not imported from epidemic country. NDM-1 was for the first time identified in *Klebsiella pneumonia* isolate in Croatia in 2008 (18) and later spread among *Enterobacteriaceae* (19). MBL-positive *Acinetobacter* spp isolates are a source of deep concern due to their multidrug resistance pattern and the ability of this genus to persist in the environment (13). Early detection of these genes will help in prevention and adequate infection control by limiting the spread of these organisms (20). Therapeutic options are often very limited particularly since colistin resistant isolates of *Acinetobacter* spp have emerged recently also in Croatia. Colistin combined with other antibiotics such as vancomycin or meropenem is recommended for the treatment of infections associated with such pandrug resistant strains (21-22)

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