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)

References

1. Bergogne-Berenzin E, Towner KJ. *Acinetobacter* spp. as nocomial pathogen: microbiological, clinical and epidemiologic features. Clin Microbiol Rev 1996;9:148-65.
2. Mammina C, Palma Dm, Bonura C, Aleo A, Fasciana T, Sodano C, Saporito MA, Verde MS, Cala C, Cracchiolo AN, Tetamo R. Epidemiology and clonality of carbapenem-resistant *Acinetobacter baumannii* from an intensive care unit in Palermo, Italy. BMC Res Notes 2012;20;5: 365

3. Goić-Barišić I, Bedenić B, Tonkić M, Katić S, Kalenić S, Punda-Polić V. Molecular characterisation of carbapenem resistant *Acinetobacter baumannii* in different Intensive Care Units in University Hospital Split, Croatia J Chemother 2007;19(4):462-464.

4. Goić-Barišić I, Bedenić B, Tonkić M, Novak A, Katić S, Kalenić S, Punda-Polić V, Towner KJ. Occurrence of OXA-107 and IS*Aba 1* in carbapenem-resistant isolates of *Acinetobacter baumannii* from Croatia. J Clin Microbiol 2009;47(10):3348-3349.

5. Goić-Barišić I, Towner, KJ, Kovačić A, Šiško-Kraljević K, Tonkić M, Novak A, Punda-Polić V. Outbreak in Croatia caused by a new carbapenem-resistant clone of *Acinetobacter baumannii* producing OXA-72 carbapenemase. J Hospit Infect 2011;77(4):368-369.

6. Franolić-Kukina I, Bedenić B, Budimir A, Herljević Z, Vraneš J, Higgins P. Clonal spread of carbapenem-resistant OXA-72 positive *Acinetobacter baumannii* in a Croatian university hospital. Int J Infect Dis 2011;15:e706-e709.

7. Vranić-Ladavac M., B. Bedenić, F. Minandri, M. Ištok, S. Frančula-Zaninović, R. Ladavac, and P. Visca. 2014. Carbapenem-resistance and acquired class D carbapenemases in *Acinetobacter baumannii* from Croatia 2009-2010. Eur. J. Clin. Microbiol. Infect. Dis. 33(3):471-8.

8. Clinical and Laboratory Standards Institute 2013. Performance Standards for Antimicrobial Susceptibility Testing; 23rd Informational Supplement, M100-S23. Wayne, PA: CLSI.

9. Bertini A., L, Poirel, P, Mugnier, J Villa, P. Nordmann, and A Caratoli. 2010. Characterization and PCR-based replicon typing of resistance plasmids in *Acinetobacter baumannii*. Antimicrobial. Agents. Chemother 54:4168-4177

10. Lauretti L, M.L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, and G.M. Rossolini. 1999.). Cloning and characterization of bla_{VIM}, a new integron-borne metallo- β-lactamase gene from *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents. Chemother. 43 (7): 1584-1590

11. Hrabak J., M. Stolobova, V. Studentova, M. Fridrichova, E. Chudackova, and H. Zemlickova. 2012. NDM-1 producing *Acinetobacter baumannii* isolated from a patient repatrieted to the Czech Republic from Egypt, July 2011. Euro. Surveill.16(17):E pub.

12. Rafei R, Dabbourssi F, Hamze M, Eveillard M, Lemarie C, Mallat H, Rolain HN, Guillou ML. First report of bla_{NDM-1} producing *Acinetobacter baumannii* isolated in Lebanon from civilians wounded during the Syrian war. Int J Infect Dis. 2014;21:21-23.

13. Revathi G, Siu K, Lu PL, Huang LY. First report of NDM-1 producing *Acinetobacter baumannii* in East Africa. Int J Infect Dis 2013;17:e1255-e1258.

14. Fallah F, Noori M, Hashemi A, Goudarzi H, Karimi A, Erfanimanesh S, Alimer S. Prevalence of bla_{NDM-1}, bla_{PER}, *bla*VEB, bla_{IMP}, and bla_{VIM} genes among *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. Scientifica (Cairo). 2014; .(Epub 2014, Juk15).

15. Bogaerts P, de Castro R, Roisin S, Deplano A, Huang TD, Hallin M. Emergence of NDM-1 producing *Acinetobacter baumannii* in Belgium. J Antimicrob Chemother 2012;67(6):1552-1553

16. Decousser W, Jansen C, Nordmann P, Emirian A, Bonnin RA, Anais L, Merie JC, Poirel L. Outbreak of NDM-1- producing *Acinetobacter baumannii* in France, January to May 2013. Eurosurveillance 2013;18 (31): pmid 20547.

17. Karthikeyen K, Thirnujan MA, Krishan P. Coexistence ob bla_{OXA-23} with bla_{NDM-1} and armA in clinical isolates of *Acinetobacter baumannii* from India. J Antimicrob Chemother 2010;65:2253-2254.

18. Mazzariol A, Bošnjak Z, Ballarini P, Budimir A, Bedenić B, Kalenić S, Cornaglia G. NDM-1 producing *Klebsiella pneumoniae*, Croatia. Emerging Infectious Diseases 2012; 18(3)532-534.

19. Zujić-Atalić V, Bedenić B, Kocsis E, Mazzariol A, Sardelić S, Barišić M, Plečko V, Bošnjak Z, Mijač M, Jajić I, Vranić-Ladavac M, Cornaglia G. Diversity of carbapenemases in clinical isolates of *Enterobacteriaceae* in Croatia-the results of the multicenter study. Clin Microbiol Infect 2014, O894-903.

20.Morfín-Otero R.[a, b] · Alcántar-Curiel M.D.[c] · Rocha M.J.[c] · Alpuche-Aranda C.M.[c] · Santos-Preciado J.I.[c] · Gayosso-Vázquez C.[c] · Araiza-Navarro J.R.[b] · Flores-Vaca M.[b] · Esparza-Ahumada S.[a, b] · González-Díaz E.[a, b] · Pérez-Gómez H.R.[a, b] · Rodríguez-Noriega E.[a, b] *Acinetobacter baumannii* Infections in a Tertiary Care Hospital in Mexico over the Past 13 Years. Chemotherapy 2013;59:57-65

21. Garnacho-Montero J. · Amaya-Villar R. · Gutiérrez-Pizarraya A. · Espejo-Gutiérrez de Tena E. · Artero-González M.L. · Corcia-Palomo Y. · Bautista-Paloma J. Clinical Efficacy and Safety of the Combination of Colistin plus Vancomycin for the Treatment of Severe Infections Caused by Carbapenem-Resistant *Acinetobacter baumannii* Chemotherapy 2013;59:225-231

22. Dinc G. · Demiraslan H. · Elmali F. · Ahmed S.S. · Metan G. · Alp E. · Doganay M. Efficacy of Sulbactam and Its Combination with Imipenem, Colistin and Tigecycline in an Experimental Model of Carbapenem-Resistant *Acinetobacter baumannii* Sepsis. Chemotherapy 2013;59:325-329