



Isolation of Oxidase-Negative *Pseudomonas aeruginosa* from Various Specimens

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Dear Editor-in-Chief

Identification of non-fermentative, gram-negative bacilli in the clinical laboratory is becoming more significant as documented evidence of pathogenicity strains against these bacteria (1). Oxidase testing is an important phenotypic method for the detection of *P. aeruginosa* and all *Pseudomonas* isolates, exception of phytopathogens are positive oxidase. In normal situations, an oxidase-producer organism does not switch into a negative strain and inversely a negative species rarely becomes a positive one (1, 2).

In the current study from Oct 2013 to Jul 2016, 371 clinical isolates were obtained from burns and general hospitals in Ahvaz, Isfahan and Tehran cities from Iran. The isolation and identification of *P. aeruginosa* were done by the conventional methods (3) and proved by PCR amplification with specific primers for *P. aeruginosa gyrB* gene (*gyrB*-F5'-CCTGACCATCCGTCGCCACAAC-3', *gyrB* R:5'CGCAGCAGGATGCCGACGCC-3' with product size 221bp). The oxidase test was performed with filter paper and oxidase test disc (1). Susceptibility of ONPA to the antibiotics was done by Kirby-Bauer's disc diffusion method. The modified Hodge test (MHT) was performed for all ONPA isolates as recommended by CLSI.

The E-test (imipenem 0.002-32µg/mL) was applied to all ONPA isolates to determine minimum inhibitory concentrations (MICs) by the manufacturer's instructions.

DNA of isolates was extracted by the DNA extraction kit (Sinaclon, Iran). The specific primers were used for different types of carbapenemase (*bla_{NDM-1}*, *bla_{IMP}*, *bla_{VIM}*, *bla_{KPC}*, *bla_{GES}*, *bla_{SPM}* and *bla_{OXA-10}*). The PCR was adjusted successfully to identify the MBL genes (*bla_{IMP}*, *bla_{VIM}*) in ONPA isolates. (Fig. 1).



Fig. 1: Gel electrophoresis of multiplex PCR products following amplification with specific primers for *bla_{IMP}* and *bla_{VIM}* gene (233 and 382 bp). Line and 13 ladder, line2 positive control *bla_{IMP}*, line 3 positive control *bla_{VIM}*, line 4 deionized water as control negative, line 5- 12 samples



Moreover, PCR assays were used for detection *bla*_{NDM-1} (Fig. 2), *bla*_{KPC}, *bla*_{GES}, *bla*_{SPM} and *bla*_{OXA-10}. The nucleotide sequences were analyzed using MEGA 6 software and blast in NCBI.

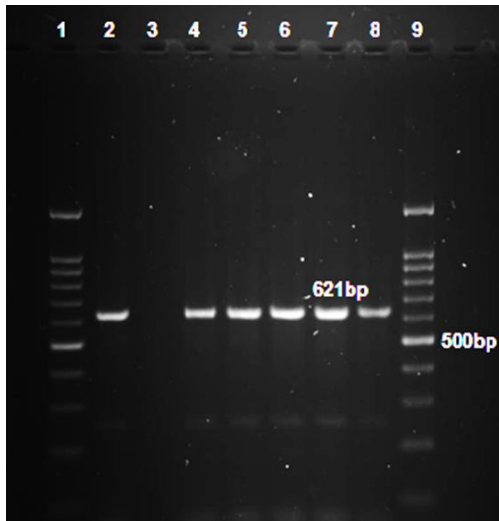


Fig. 2: Gel electrophoresis of PCR products following amplification with specific primers for *bla*_{NDM-1} gene (621 bp). Line 1 and 9 ladder, line 2 *bla*_{NDM-1} positive control (ATCC: BAA-2146), line 2 deionized water as control negative, line 3-7 positive samples

Among 371 *P. aeruginosa* isolates, 26 (7%) were ONPA isolates that 5 out of 26 (19.2%) ONPA isolates were found carrying *bla*_{NDM-1} as confirmed by sequencing. Among 26 ONPA isolates 13 were completely resistant to imipenem, meropenem and ertapenem and 16 were positive for carbapenemase production by MHT and ten those isolates were shown to have MBL genes. Among these isolates high-level resistance to cefotaxime, ertapenem and meropenem was observed. All of isolates were susceptible to colistin. In ONPA isolates, *bla*_{IMP-1}, *bla*_{VIM-2} and *bla*_{OXA-10} were identified in 9 (34.6%), 2 (7.7%) and 14 (53.7%) of isolates respectively, while co-producing *bla*_{NDM-1}, *bla*_{IMP-1}, *bla*_{OXA-10} and co-producing *bla*_{NDM-1}, *bla*_{IMP-1}, *bla*_{VIM-2} and *bla*_{OXA-10} were determined in three and one of ONPA isolates. *bla*_{KPC}, *bla*_{GES}, and *bla*_{SPM} genes were not detected.

We report the presence of *bla*_{NDM-1} in ONPA clinical isolates in a university teaching hospital in Iran. To date, no reports of ONPA isolates co-existing *bla*_{NDM-1} or other carbapenemase genes in patients. In addition, finding ONPA is warning about the mistake in the laboratory diagnosis. For the first time, in 1979, Kenneth D et al have reported two uncommon isolates with negative oxidase test obtained from a sputum sample. (4). Followed Ferne K et al. one year later describe an isolate of ONPA collected from a catheterized urine specimen in Texas (5). NDM enzymes are one of the greatest important emerging resistance elements to carbapenem antibiotics. Resistance to carbapenems has led to global concerns because carbapenems are considered as the last effective drug of choice for *P. aeruginosa* infections (6). There are only few reports in the literature of *P. aeruginosa* co-producing three carbapenemases (7). In this study, we reported three isolates harboring three carbapenemases and one isolate harboring four carbapenemases.

Since the oxidase test is essential for the diagnosis of *P. aeruginosa*, the finding and emerging ONPA of this co-existence of multiple carbapenem resistance genes are warning about the mistake in the laboratory diagnosis. The results showed the serious therapeutic risk of MBL-producing ONPA societies. The appearance of ONPA carrying *bla*_{NDM-1} and other carbapenemase genes is indicating great challenge in the treatment. This information can help to generate the proper strategies for this purpose.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Amjad A, Mirza I, Abbasi S et al (2011). Modified Hodge test: A simple and effective test for detection of carbapenemase production. *Iran J Microbiol*, 3(4):189-193.
2. Bahar MA, Jamali S, Samadikuchaksaraei A (2010). Imipenem-resistant *Pseudomonas*

- aeruginosa strains carry metallo- β -lactamase gene bla VIM in a level I Iranian burn hospital. *Burns*, 36(6):826-830.
3. Luce E. Koneman's color atlas and textbook of diagnostic microbiology. *Plast Reconstr Surg*;125(1):414-5.
 4. Hampton KD, Wasilaukas BL (1979). Isolation of oxidase-negative *Pseudomonas aeruginosa* from sputum culture. *J Clin Microbiol*, 9(5):632-634.
 5. McCleskey FK, Adams Jr ED (1980). Isolation of oxidase-negative *Pseudomonas aeruginosa* from urine culture. *J Clin Microbiol*, 12(4):624-25.
 6. Jamal WY, Albert MJ, Rotimi VO (2016). High Prevalence of New Delhi Metallo- β -Lactamase-1 (NDM-1) Producers among Carbapenem-Resistant Enterobacteriaceae in Kuwait. *PLoS One*, 11(3): e0152638.
 7. Wang M, Borris L, Aarestrup FM, Hasman H (2015). Identification of a *Pseudomonas aeruginosa* co-producing NDM-1, VIM-5 and VIM-6 metallo- β -lactamases in Denmark using whole-genome sequencing. *Int J Antimicrob Agents*, 45(3):324-325.