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Detection of bla_{SPM-1} and bla_{SIM-2} Metallo-β-Lactamases Genes in Imipenem-Resistant *Pseudomonas aeruginosa* Clinical Isolates Recovered from Two University Hospitals in Egypt

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ABSTRACT

Objectives: Unraveling mechanisms whereby *Pseudomonas aeruginosa* becomes resistant to carbapenems through testing 114 non-duplicate *P. aeruginosa* clinical isolates for their susceptibility to various classes of antibiotics and scrutinizing the production of metallo-β-lactamases (MBLs) by tested isolates. **Methods:** Susceptibility testing of *P. aeruginosa* to different antibiotics was determined by Kirby-Bauer disk diffusion method and MBLs production by tested isolates was studied phenotypically and genotypically and PCR products were confirmed by sequencing **Results:** All tested clinical isolates showed eminent resistance to the majority of tested antibiotics and 14 isolates were imipenem (IPM)-resistant. Furthermore, IPM-resistant isolates were verified to be MBLs-producers. MBLs-encoding genes bla_{SIM} and bla_{SPM} genes were detected by PCR where four isolates were found to harbor bla_{SPM} gene while only one isolate harbored bla_{SIM} gene. The correct size of PCR products of bla_{SIM} and bla_{SPM} genes were sequenced and sequences were submitted to the GenBank databases and assigned the accession numbers **KX452682** and **KX452683** for bla_{SIM-2} and bla_{SPM-1}, respectively. **Conclusion:** Here, we report the emanation of *P. aeruginosa* clinical isolates harboring bla_{SPM-1} and bla_{SIM-2} genes. This may reflect the substantial increase in the rate of imipenem resistance due to MBL in *P. aeruginosa* clinical isolates from Egypt. Early detection and infection-control practices are of the best antimicrobial strategy for combating this organism.

Keywords: PCR; MDR; Sequencing; Antimicrobial susceptibility tests; Carbapenemase

INTRODUCTION

Pseudomonas aeruginosa, a notorious primary opportunistic pathogen, is the leading cause of hospital-acquired infections worldwide and commonly associated with high fatality and morbidity rates[1]. Among the most common Gram negative bacteria, *P. aeruginosa* is the most frequently isolated worrisome

pathogen that causes life threatening respiratory tract infection, urinary tract infections in patients admitted to intensive care units[2,3]. Infections caused by this pathogen are often difficult to be treated because of both its intrinsic resistance and its remarkable ability to acquire further resistance mechanisms to a plethora of antibiotics[4,5]. Despite improvements in antibiotic therapy, there is still an escalating difficulty in finding new antibiotics and search for alternative approaches to combat drug resistance[1,6].

The acquired resistance of *P. aeruginosa* to beta lactam antibiotics has been attributed to several mechanisms including β-lactamase production, upregulation of efflux systems and reduced outer membrane permeability[7,8]. However, acquired extended spectrum beta lactamases (ESBL) and metallo-β-lactamases (MBLs)-mediated resistance are the most important emerging mechanisms of resistance in *P. aeruginosa* since carbapenemases often jeopardize chemotherapy for infectious diseases[8]. Despite the abundant literature on those mechanisms in *P. aeruginosa*, there is no data regarding the prevalence of such novel MBLs (especially *bla*$_{SIM}$ and *bla*$_{SPM}$) harbored by *P. aeruginosa* in Egypt.

MBLs are particularly worrisome in the clinical setting due to their broad range, robust carbapenemases activity, and resistance to β-lactamase inhibitors[9]. Various types of MBLs have been identified so far, including Imipenemase (IMP), Verona integron-encoded metallo-β-lactamase (VIM), Sao Paulo metallo-β-lactamase (SPM), Germany imipenemase (GIM), New Delhi metallo-β-lactamase (NDM) and Florence imipenemase (FIM)[10-14].

The ultimate goal of current work was to study the susceptibility of *P. aeruginosa* clinical isolates (obtained from two University hospitals in Egypt) to the most common clinically-used antibiotics including carbapenems and to investigate the production of MBLs (*bla*$_{SIM}$ and *bla*$_{SPM}$ by these isolates.

MATERIAL AND METHODS

Bacterial strains, antibiotic susceptibility testing and MIC determination

Bacterial isolates were recovered from inpatients hospitalized in two main referral hospitals at Tanta and Mansoura universities. A total of 114 consecutive, clinical samples were collected from various sources including urine (n=38), wound swab (n=14), sputum (n=20) and blood (n=42) using proper sampling techniques[15,16] and inoculated onto Pseudomonas P agar. Susceptibility of *P. aeruginosa* to various classes of antibiotics was tested by Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute guidelines (CLSI-2014)[17] and were ascertained by automated methods

using VITEK 2 Compact System[18]. The tested antibiotics included imipenem, meropenem, ticarcillin, ticarcillin/clavulanic acid, ciprofloxacin, tobramycin, ceftazidime, piperacillin, piperacillin /tazobactam and colistin.

The MIC values of the aforementioned 10 antimicrobial agents for 114 isolates were determined as described by Clinical and Laboratory Standard Institute-CLSI [17]. *P. aeruginosa* ATCC 27853 was used as the control reference strain.

Molecular detection and sequencing of MBLs genes in *P. aeruginosa* isolates

The genomic DNA was extracted from selected isolates using Gene JET genomic DNA extraction kit following the manufacturer instructions (Fermentas, #K0721). The extracted DNA was subjected to PCR for the detection the MBLs genes namely, *bla*$_{SPM}$ and *bla*$_{SIM}$ using specific primer sets targeting those genes as previously described[19]. The primers sequences used were: *bla*$_{SPM}$ gene forward primer 5'AAAATCTGGGTACGCAAACG3', reverse primer 5'ACATTATCCGCTGGAACAGG3' and *bla*$_{SIM}$ gene forward primer 5'TAC-AAGGGATTCGG CATCG3', reverse primer 5'TAATGGCCTGTT CCCATGTG3'.

The PCR reaction was performed in a final volume of 50 µl comprising 5 µl of DNA template (5-20 ng/µl); 25 µl of PCR master mix; 2.5 µl of forward primer (0.1-0.5 μM); 2.5 µl of reverse primer (0.1-0.5 μM) and 15 µl nuclease-free water and the DNA was amplified using thermal cycler (Applied Biosystem, USA). The amplification was carried out using the following protocol: initial denaturation (95°C, 5 min), followed by 35 cycles of denaturation (94°C, 30s), annealing (52°C, 40s), extension (72°C, 50s) and final cycle of amplification (72°C, 5min). Positive and a negative controls for each PCR were included. The DNA fragments were separated by agarose gel electrophoresis and amplified products were visualized and analyzed under UV light. *P. aeruginosa* ATCC 27853 was used as the control strain.

Samples were purified using PCR purification kit (Jena Bioscience, # PP-201S, Germany) according to the manufacturer's catalogue[37]. The purified PCR products were sent to MacroGen Company (South Korea) for sequencing (ABI 3730XL DNA sequencer, Applied Biosystem, USA). The Sequences were analyzed using the ChromasLite 2.1 program (http://technelysium.com.au/?page_id=13) and the identity of the sequenced PCR products were investigated using Blast search against Genbank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The alignments, annotations and assembly of the sequences were performed using Geneious 4.8.4 software http://www.geneious.com/web/geneious/home.

Table 1. Antibiogram of tested *P. aeruginosa* isolates

Antimicrobial agent	No of sensitive isolates	MIC for sensitive isolates (mg/L)	No of resistant isolates	MIC for resistant isolates (mg/L)
Ciprofloxacin	64	≤1	50	≥8
Tobramycin	95	≤4	19	≥32
Imipenem	100	≤1	14	≥16
Meropenem	105	≤1	9	≥16
Ceftazidime	59	≤4	55	≥64
Ticarcillin	84	≤8	30	≥256
Ticarcillin/ Clav	84	≤8	30	≥256
Piperacillin	89	≤4	25	≥256
piperacillin/tazobactam	90	≤4	24	≥256
Colistin	114	≤1	0	-------

Statistical analyses

Pearson $\chi2$ or Fisher's exact tests were performed to compare the number of isolates resistant and sensitive to antibiotics. Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA); $P \leq 0.05$ was considered as level of significance for the two-tailed test.

RESULTS

Identification of clinical isolates and antimicrobial susceptibility testing

A total of 114 non-duplicate clinical isolates of *P. aeruginosa*, collected from different clinical sources, were identified based on their morphological, cultural and biochemical characteristics as well as by automated VITEK2 system. Susceptibility of all tested *P. aeruginosa* clinical isolates to the 10 tested antibiotics was examined and tested isolates were then classified according to their resistance profile into XDR and MDR. Totally, 43.8% (n=50) of the isolates were MDR, 17.5% (n=20) were XDR and 12.3% (n=14) were Imipenem-resistant (MIC>8mg/L). Interestingly, colistin was the most effective antibiotic (no resistant isolates were detected). MIC values obtained for all tested antibiotics are listed in Table 1 and the prevalence of bla_{SIM} and bla_{SIM} genes among XDR, MDR and IPM-resistant isolates is shown in Table 2.

Molecular detection of MBLs encoding genes
Isolation of Bacterial DNA

DNA was extracted from *P. aeruginosa* clinical isolates as revealed by gel electrophoresis which showed intact bands indicating pure genomic DNA. The DNA concentration and purity were determined by Nanodrop (uv-vis spectrophotometer Q5000, Quawell, USA) by measuring the absorption at 260 and 280 nm. The ratio of 260/280 and 230/260 ranged between 1.8 and 2.2 confirming the purity of extracted DNA.

Detection of MBLs encoding genes

To confirm phenotypic results, PCR was done to detect MBLs encoding genes. PCR amplification and sequencing confirmed the presence of bla_{SIM} gene in one isolate and bla_{SPM} in 4 isolates out of 114 *P. aeruginosa* isolates. Of note, one MDR isolate was found to harbour both bla_{SIM} and bla_{SPM}.

The MBLs-encoding bla_{SIM} and bla_{SPM} genes that were detected in the current study were consistent with those of the positive controls with PCR products matching the predicted sizes of bla_{SIM}= 570bp and bla_{SPM}= 271bp. PCR results revealed 4 isolates harbored bla_{SPM} gene and only one isolate harbored bla_{SIM} gene. Data obtained also revealed that bla_{SIM} and bla_{SPM} genes were detected in isolates collected from blood samples only.

Table 2. Prevalence of *bla*SIM-2 and *bla*SPM-1 genes among isolates and their related antimicrobial resistance profile

MBL gene	Prevalence among all isolates	Prevalence among MDR-isolates	Prevalence among IPM-resistant MDR-isolates	Prevalence among XDR-isolates	Prevalence among IPM-resistant XDR-isolates
*bla*SIM-2	1/114 (0.8%)	1/20 (5%)	1/8 (12.5%)	0/14 (0%)	0/6 (0%)
*bla*SPM-1	4/114 (3.5 %)	2/20 (10%)	2/8 (25%)	2/14 (14%)	2/6 (33.3%)

IPM; imipenem, MDR; multiple drug resistant and XDR; extensively drug resistant
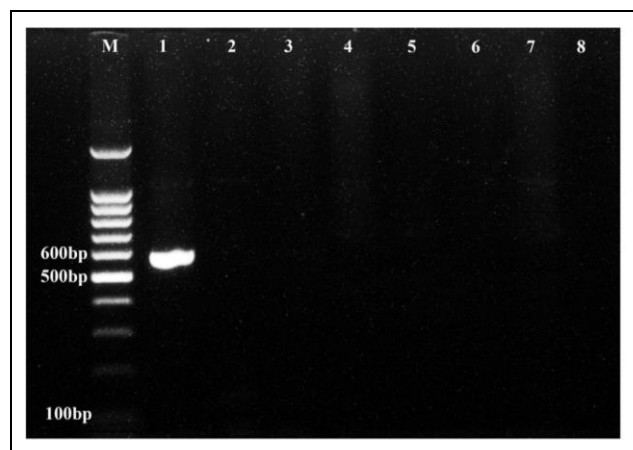

Figure 1. Ethidium bromide stained gel shows PCR products of *bla*$_{SIM}$ gene with size of 570bp in one positive isolate (lane 1); negative isolates (lanes 2-8);M100 bp marker (ladder).
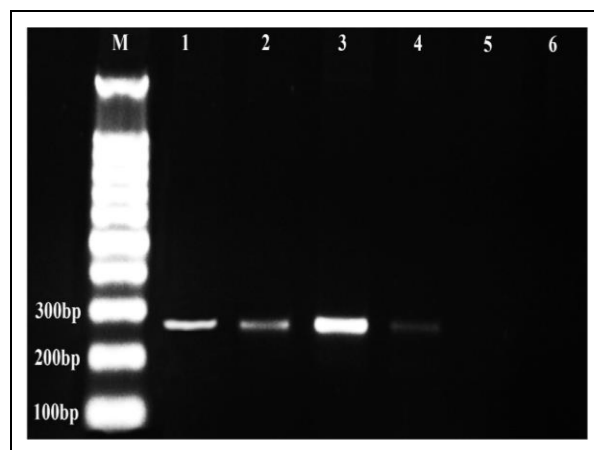

Figure 2. Ethidium bromide stained gel shows PCR products of *bla*$_{SPM}$ gene with size of 271bp in four positive isolates (lanes 1, 2, 3 and 4); negative isolates (lanes 5 and 6); M 100bp marker (ladder).

DNA sequencing and alignments of MBLs encoding genes

PCR products of MBLs genes [*bla*$_{SPM}$ and *bla*$_{SIM}$] were sequenced in both directions to search for any potential polymorphism among MBLs genes of tested isolates. These sequences were submitted to the GenBank databases and assigned the accession numbers **KX452682 and KX452683** for *bla*$_{SIM-2}$ and *bla*$_{SPM-1}$, respectively. Data analysis of these sequences showed 100% identity of nucleotide sequences of MBLs encoding genes among tested Egyptian isolates (see supplementary data).

DISCUSSION

P. aeruginosa is a notorious pathogen that poses a serious problem to the human health sector[20]. This organism is mainly associated with multiple dangerous disorders including ventilator-associated pneumonia, urinary tract infections, meningitis, cystic fibrosis, catheter-associated infections, corneal infections and soft tissue infections[21].

The emanation of MBLs-mediated carbapenems resistance in *P. aeruginosa* and dissemination of carbapenemases via horizontal gene transfer represent a major rising public health hazard[22]. Globally, there is a substantial increase in MBLs production by carbapenem-resistant *P. aeruginosa*. In recent years, Egypt was among the countries that reported high rates of antimicrobial resistance[23], however, data regarding the prevalence of MBLs (*bla*$_{SIM}$ and *bla*$_{SPM}$) production by *P. aeruginosa* isolates in Egypt is so limited [24,25]. Therefore, this work aimed at studying the prevalence of MBLs-producing *P. aeruginosa* clinical isolates from Egypt. Investigating the MBLs production by *P. aeruginosa* is a crucial step towards large scale monitoring of these emerging resistant determinants and could pave the way towards finding new interventions to the threatening problem of massive emergence of MDR pathogens.

Although comparison between studies is difficult since the patient populations of the centers and the methods of studying differ, here we reported 14 IPM-resistant *P. aeruginosa* (12%) which was higher (5.7%) than that was previously published in Iran[26]. On contrary, another study from Iran reported a higher prevalence percentage (51.4%) of IPM-resistant *P. aeruginosa* [27].

Colistin belongs to the polymyxin family and has a wide range of activity against most clinically-relevant Gram-negative bacteria. Colistin is currently used for clinical application and represents the mainstay and the last resort for treatment of carbapenem-resistant *P. aeruginosa*. Based on the breakpoint reported by CLSI[28] for colistin against *P. aeruginosa*, a susceptible breakpoint of $\leq$ 2mg/L and a resistant breakpoint of $\geq$ 4mg/L were applied to all isolates. Interestingly, colistin was the most effective antimicrobial agent in study since all of our tested isolates (n=114) were found to be sensitive to colistin.

Various MBLs have previously been reported in Egypt including bla_{NDM-1} in Gram negative bacteria[29], bla_{NDM-2} in *Acinetobacter baumannii*[30], bla_{OXA-48} and bla_{VIM-1} in Enterobacteriaceae[31], bla_{OXA-48} and bla_{NDM-1} in *Klebsiella pneumoniae*[32], bla_{VIM-4} in *Enterobacter cloacae*[33], bla_{NDM-1} in *P. aeruginosa* [25], bla_{NDM-1} in *Klebsiella pneumoniae*[34,35], bla_{GIM} in *P. aeruginosa*[36], bla_{VIM-2} in *P. aeruginosa*[24], bla_{VIM-28} in *P. aeruginosa*[37], bla_{IMP} and bla_{VIM} in *P. aeruginosa*[38] and bla_{GIM} in *Acinetobacter baumannii*[39]. Very recently, metallo-beta-lactamases producing-Pseudomonas aeruginosa were isolated from hospitals in Minia, Egypt[40]. Furthermore, a recent work from our laboratory has shown the emergence of *P. aeruginosa* clinical isolates that coproduce bla_{VIM} and bla_{IMP} carbapenemases[41]. Moreover, we have recently reported the emergence of Imipenem-resistant *Pseudomonas aeruginosa* clinical isolates from Egypt that harbor bla_{GIM-1} gene[42].

The MBL gene bla_{SIM} was first identified in *Acinetobacter baumannii*[43]. Our data showed the emergence of one bla_{SIM-2}-producing *P. aeruginosa* clinical isolate out of 114 total isolates with a prevalence of (0.8%). As per literature, bla_{SIM}-producing *P. aeruginosa* was previously reported in China[44]. Further investigations are required to identify possible reasons whereby bla_{SIM} gene was transferred to Egypt. Sequence analysis showed 100% identity with the bla_{SIM} gene of *P. aeruginosa* (accession number **KX452682**).

In 1997, bla_{SPM}-producing *P. aeruginosa* was first isolated in Brazil[45]. Quite later, bla_{SPM}-producing *P. aeruginosa* was detected in Europe for patients who had a pervious history of travel to Brazil[46,47]. Here, we reported bla_{SPM-1}-*P. aeruginosa* clinical isolates with a prevalence of (1.75%) compared to (17%) in Brazil[48]. Sequence analysis showed 100% identity with the bla_{SPM} gene of *P. aeruginosa* (accession number **KX452683**).

Interestingly, the DNA sequence analysis of our bla_{SPM} and bla_{SIM} showed 100% identity with the sequence of global genotypes. The results, further confirmed that, MBL genes might have been evolved from the same clone and can spread to another population by selective pressure of antibiotic which is described in that local region.

Therefore, the periodic investigation of antibiotic resistance profiles and the resistance genes among clinical *P. aeruginosa* strains in Egypt is so crucial to gauge the level of activity among commonly prescribed antipseudomonal drugs. Moreover, local epidemiology patterns need to be actively evaluated as the majority of endemic cases usually occurs through the clonal dissemination of notorious MBLs-producing clones.

CONCLUSION

Altogether, the present study reports the emanation of bla_{SIM-2}- and bla_{SPM-1}-producing *P. aeruginosa* in Egypt which raises the concern about its potential spread to other pathogens infecting hospitalized patients.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. El, Z.M.E.; Gyetvaii, B. Effectiveness of Antipseudomonal Antibiotics and Mechanisms of Multidrug Resistance in *Pseudomonas aeruginosa*. *Pol. J. Microbiol*. **2016**, *65* (1), 23-32.

2. Yan, Y.; Yao, X.; Li, H.; Zhou, Z.; Huang, W.; Stratton, C.W.; Lu, C.D.; Tang, Y.W. A novel *Pseudomonas aeruginosa* strain with an oprD mutation in relation to a nosocomial respiratory infection outbreak in an intensive care unit. *J. Clin. Microbiol*. **2014**, *52* (12), 4388-90.

3. Riou, M.; Carbonnelle, S.; Avrain, L.; Mesaros, N.; Pirnay, J.P.; Bilocq, F.; De Vos, D.; Simon, A.; Pierard, D.; Jacobs, F.; Dediste, A.; Tulkens, P.M.; Van Bambeke, F.; Glupczynski, Y. In vivo development of antimicrobial resistance in Pseudomonas aeruginosa strains isolated from the lower respiratory tract of Intensive Care Unit patients with nosocomial pneumonia and receiving antipseudomonal therapy. *Int. J. Antimicrob. Agents*. **2010**, *36* (6), 513-22.

4. Johansen, H.K.; Moskowitz, S.M.; Ciofu, O.; Pressler, T.; Hoiby, N. Spread of colistin resistant non-mucoid *Pseudomonas aeruginosa* among

chronically infected Danish cystic fibrosis patients. *J. Cyst. Fibros*. **2008**, *7* (5), 391-7.

5. Bergen, P.J.; Bulitta, J.B.; Forrest, A.; Tsuji, B.T.; Li, J.; Nation, R.L. Pharmacokinetic/ pharmacodynamic investigation of colistin against *Pseudomonas aeruginosa* using an in vitro model. *Antimicrob. Agents. Chemother*. **2010**, *54* (9), 3783-9.

6. Hamed, S.; Emara, M.; Shawky, R.M.; El-Domany, R.A.; Youssef, T. Silver nanoparticles: Antimicrobial activity, cytotoxicity, and synergism with N-acetyl cysteine. *J. Basic. Microbiol*. **2017**, *57* (8), 659-668.

7. Zavascki, A.P.; Carvalhaes, C.G.; Picao, R.C.; Gales, A.C. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: resistance mechanisms and implications for therapy. *Expert. Rev. Anti Infect. Ther*. **2010**, *8* (1), 71-93.

8. Shen, J.L.; Zhu, D.M.; Wang, M.G. Mechanisms of pandrug-resistance of *Pseudomonas aeruginosa*. *Zhonghua Yi Xue Za Zhi*. **2008**, 88 (26), 1859-62.

9. Tada, T.; Miyoshi-Akiyama, T.; Shimada, K.; Shimojima, M.; Kirikae, T. IMP-43 and IMP-44 metallo-beta-lactamases with increased carbapenemase activities in multidrug-resistant *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother*. **2013**, *57* (9), 4427-32.

10. Jovcic, B.; Lepsanovic, Z.; Suljagic, V.; Rackov, G.; Begovic, J.; Topisirovic, L.; Kojic, M. Emergence of NDM-1 metallo-beta-lactamase in *Pseudomonas aeruginosa* clinical isolates from Serbia. *Antimicrob. Agents Chemother*. **2011**, *55* (8), 3929-31.

11. Lee, K.; Lim, J.B.; Yum, J.H.; Yong, D.; Chong, Y.; Kim, J.M.; Livermore, D.M. bla(VIM-2) cassette-containing novel integrons in metallo-beta-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. *Antimicrob. Agents Chemother*. **2002**, *46* (4), 1053-8.

12. Poirel, L.; Nordmann, P.; Lagrutta, E.; Cleary, T.; Munoz-Price, L.S. Emergence of KPC-producing *Pseudomonas aeruginosa* in the United States. *Antimicrob Agents Chemother*. **2010**, *54* (7), 3072.

13. Martins, A.F.; Zavascki, A.P.; Gaspareto, P.B.; Barth, A.L. Dissemination of Pseudomonas aeruginosa producing SPM-1-like and IMP-1-like metallo-beta-lactamases in hospitals from southern Brazil. *Infection* **2007**, *35* (6), 457-60.

14. Sevillano, E.; Gallego, L.; Garcia-Lobo, J.M. First detection of the OXA-40 carbapenemase in *P. aeruginosa* isolates, located on a plasmid also found in A. baumannii. *Pathol. Biol. (Paris)*. **2009**, *57* (6), 493-5.

15. Cheesbrough, M. District laboratory practice in tropical countries. Cambridge University Press, London **2006**, pp. 319–321.

16. Vandepitte, J.; Engbaek, K.; Piot, P.; Heuck, C.C.; Rohner, P. 2003. Basic Laboratory Procedures in Clinical Bacteriology Paperback. 2nd ed. WHO.

17. Wayne, P. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI M100-S24. *Clinical and Laboratory Standards Institute*. **2014**, M100-S24

18. de Cueto, M.; Ceballos, E.; Martinez-Martinez, L.; Perea, E.J.; Pascual, A. Use of positive blood cultures for direct identification and susceptibility testing with the vitek 2 system. *J. Clin. Microbiol*. **2004**, *42* (8), 3734-8.

19. Ellington, M.J.; Kistler, J.; Livermore, D.M.; Woodford, N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. *J. Antimicrob. Chemother*. **2007**, 59 (2), 321-2.

20. Fournier, A.; Voirol, P.; Krahenbuhl, M.; Bonnemain, C.L.; Fournier, C.; Pantet, O.; Pagani, J.L.; Revelly, J.P.; Dupuis-Lozeron, E.; Sadeghipour, F.; Pannatier, A.; Eggimann, P.; Que, Y.A. Antibiotic consumption to detect epidemics of Pseudomonas aeruginosa in a burn centre: A paradigm shift in the epidemiological surveillance of Pseudomonas aeruginosa nosocomial infections. *Burns* **2016**, *42* (3), 564-70.

21. Williamson, K.S.; Richards, L.A.; Perez-Osorio, A.C.; Pitts, B.; McInnerney, K.; Stewart, P.S.; Franklin, M.J. Heterogeneity in *Pseudomonas aeruginosa* biofilms includes expression of ribosome hibernation factors in the antibiotic-tolerant subpopulation and hypoxia-induced stress response in the metabolically active population. *J. Bacteriol*. **2012**, *194* (8), 2062-73.

22. Bush, K.; Pannell, M.; Lock, J.L.; Queenan, A.M.; Jorgensen, J.H.; Lee, R.M.; Lewis, J.S.; Jarrett, D. Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. *Int. J. Antimicrob. Agents*. **2013**, *41* (1), 1-4.

23. Mahmoud, A.; Zahran, W.; Hindawi, G.; Labib, A.; Galal, R. Prevalence of Multidrug-Resistant *Pseudomonas aeruginosa* in Patients with Nosocomial Infections at a University Hospital in Egypt, with Special Reference to Typing Methods. *J. Virol. Microbiol*. **2013**, 1-13.

24. Zafer, M.M.; Al-Agamy, M.H.; El-Mahallawy, H.A.; Amin, M.A.; El Din Ashour, S. Dissemination of VIM-2 producing *Pseudomonas aeruginosa* ST233 at tertiary care hospitals in Egypt. *BMC Infect. Dis*. **2015**, *15*, 122.

25. Zafer, M.M.; Amin, M.; El Mahallawy, H.; Ashour, M.S.; Al Agamy, M. First report of NDM-1-

producing *Pseudomonas aeruginosa* in Egypt. *Int. J. Infect. Dis*. **2014**, 29, 80-1.

26. Ghamgosha, M.; Shahrekizahedani, S.; Kafilzadeh, F.; Bameri, Z.; Taheri, R.A.; Farnoosh, G. Metallo-beta-Lactamase VIM-1, SPM-1, and IMP-1 Genes Among Clinical *Pseudomonas aeruginosa* Species Isolated in Zahedan, Iran. *Jundishapur. J. Microbiol*. **2015**, *8* (4), e17489.

27. Moosavian, M.; Rahimzadeh, M. Molecular detection of metallo-beta-lactamase genes, bla IMP-1, bla VIM-2 and bla SPM-1 in imipenem resistant *Pseudomonas aeruginosa* isolated from clinical specimens in teaching hospitals of Ahvaz, Iran. *Iran J. Microbiol*. **2015**, *7* (1), 2-6.

28. CLSI. Clinical and Laboratory Standards Institute**.** Performance standards for antimicrobial susceptibility testing; 24th informational supplement.CLSI M100-S24. *Clinical and Laboratory Standards Institute, Wayne, PA*. **2014**, M100-S24

29. Wassef, M.; Abdelhaleim, M.; Ghaith, D.; El-Mahdy, Y. Emerging New Delhi metallo-beta-lactamase-1-type-producing Gram-negative bacteria isolated from Cairo University Pediatric Hospital, Cairo, Egypt. *J. Glob. Antimicrob. Resist*. **2016**, *7*, 84-87.

30. Kaase, M.; Nordmann, P.; Wichelhaus, T.A.; Gatermann, S.G.; Bonnin, R.A.; Poirel, L. NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. *J. Antimicrob. Chemother*. **2011**, *66* (6), 1260-1262.

31. Poirel, L.; Abdelaziz, M.O.; Bernabeu, S.; Nordmann, P. Occurrence of OXA-48 and VIM-1 carbapenemase-producing Enterobacteriaceae in Egypt. *Int. J. Antimicrob. Agents*. **2012**, *41* (1), 90-101.

32. Ghaith, D.M.; Zafer, M.M.; Said, H.M.; Elanwary, S.; Elsaban, S.; Al-Agamy, M.H.; Bohol, M.F.F.; Bendary, M.M.; Al-Qahtani, A.; Al-Ahdal, M.N. Genetic diversity of carbapenem-resistant *Klebsiella Pneumoniae* causing neonatal sepsis in intensive care unit, Cairo, Egypt. *Eur. J. Clin. Microbiol. Infect Dis*. **2019**, *39* (3), 583-591.

33. Dimude, J.U.; Amyes, S.G. Molecular characterisation and diversity in *Enterobacter cloacae* from Edinburgh and Egypt carrying bla(CTX-M-14) and bla(VIM-4) beta-lactamase genes. *Int. J. Antimicrob. Agents*. **2013**, *41* (6), 574-577.

34. Khalil, M.A.F.; Elgaml, A.; El-Mowafy, M. Emergence of Multidrug-Resistant New Delhi Metallo-beta-Lactamase-1-Producing *Klebsiella pneumoniae* in Egypt. *Microb. Drug Resist*. **2016**, *23* (4), 480-487.

35. Mohamed, E.R.; Ali, M.Y.; Waly, N.; Halby, H.M.; El-Baky, R.M.A. The Inc FII Plasmid and its

Contribution in the Transmission of blaNDM-1 and blaKPC-2 in *Klebsiella pneumoniae* in Egypt. *Antibiotics (Basel)*. **2019**, *8* (4), 266.

36. Hashem, H.; Hanora, A.; Abdalla, S.; Shaeky, A.; Saad, A. Dissemination of metallo-beta-lactamase in *Pseudomonas aeruginosa* isolates in Egypt: mutation in blaVIM-4. *APMIS*. **2017**, *125* (5), 499-505.

37. El-Mahdy, T.S. Identification of a novel metallo-beta-lactamase VIM-28 located within unusual arrangement of class 1 integron structure in *Pseudomonas aeruginosa* isolates from Egypt. *Jnp. J. Infect. Dis*. **2014**, *67* (5), 382-4.

38. Abaza, A.F.; El Shazly, S.A.; Selim, H.S.A.; Aly, G.S.A. Metallo-Beta-Lactamase Producing *Pseudomonas aeruginosa* in a Healthcare Setting in Alexandria, Egypt. *Pol. J. Microbiol*. **2018**, *66* (3), 297-308.

39. Alkasaby, N.M.; El Sayed Zaki, M. Molecular Study of *Acinetobacter baumannii* Isolates for Metallo-beta-Lactamases and Extended-Spectrum-beta-Lactamases Genes in Intensive Care Unit, Mansoura University Hospital, Egypt. *Int. J. Microbiol*. **2017**, *2017*, 3925868.

40. Farhan, S.M.; Ibrahim, R.A.; Mahran, K.M.; Hetta, H.F.; Abd El-Baky, R.M. Antimicrobial resistance pattern and molecular genetic distribution of metallo-beta-lactamases producing *Pseudomonas aeruginosa* isolated from hospitals in Minia, Egypt. *Infect. Drug Resist*. **2019**, *12*, 2125-2133.

41. El-Domany, R.A.; Emara, M.; El-Magd, M.A.; Moustafa, W.H.; Abdeltwab, N.M. Emergence of Imipenem-Resistant *Pseudomonas aeruginosa* Clinical Isolates from Egypt Coharboring VIM and IMP Carbapenemases. *Microb Drug Resist (In press)*. **2017**,

42. Abdeltwab, N.M.; Emara, M.; El-Mahdy, T.S.; El-Magd, M.A.; Moustafa, W.H.; El-domany, R.A. First report of Imipenem-resistant *Pseudomonas aeruginosa* clinical isolates harboring blaGIM-1 gene in the middle east region. *N. Egypt J. Microbiol*. **2019**, Vol. 52

43. Lee, K.; Yum, J.H.; Yong, D.; Lee, H.M.; Kim, H.D.; Docquier, J.D.; Rossolini, G.M.; Chong, Y. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. *Antimicrob. Agents Chemother*. **2005**, *49* (11), 4485-91.

44. Sun, F.; Zhou, D.; Wang, Q.; Feng, J.; Feng, W.; Luo, W.; Zhang, D.; Liu, Y.; Qiu, X.; Yin, Z.; Chen, W.; Xia, P. The first report of detecting the blaSIM-2 gene and determining the complete sequence of the SIM-encoding plasmid. *Clin. Microbiol. Infect*. **2016**, *22* (4), 347-51.

45. Toleman, M.A.; Simm, A.M.; Murphy, T.A.; Gales, A.C.; Biedenbach, D.J.; Jones, R.N.; Walsh, T.R. Molecular characterization of SPM-1, a novel metallo-beta-lactamase isolated in Latin America: report from the SENTRY antimicrobial surveillance programme. *J. Antimicrob. Chemother*. **2002**, *50* (5), 673-9.

46. Hopkins, K.L.; Meunier, D.; Findlay, J.; Mustafa, N.; Parsons, H.; Pike, R.; Wright, L.; Woodford, N. SPM-1 metallo-beta-lactamase-producing *Pseudomonas aeruginosa* ST277 in the UK. *J. Med. Microbiol*. **2016**, *65* (7), 696-7.

47. Salabi, A.E.; Toleman, M.A.; Weeks, J.; Bruderer, T.; Frei, R.; Walsh, T.R. First report of the metallo-beta-lactamase SPM-1 in Europe. *Antimicrob. Agents Chemother*. **2009**, 54 (1), 582.

48. Furtado, G.H.; Gales, A.C.; Perdiz, L.B.; Santos, A.F.; Medeiros, E.A. Prevalence and clinical outcomes of episodes of ventilator-associated pneumonia caused by SPM-1-producing and non-producing imipenem-resistant *Pseudomonas aeruginosa*. *Rev. Soc. Bras. Med. Trop*. **2011**, *44* (5), 604-606.