Bulgarian Journal of Veterinary Medicine, 2021, **24**, No 2, 200–207
ISSN 1311-1477; DOI: 10.15547/bjvm.2019-0070

Original article

EVALUATION OF MexAB-OprM EFFLUX PUMP AND DETERMINATION OF ANTIMICROBIAL SUSCEPTIBILITY IN *PSEUDOMONAS AERUGINOSA* HUMAN AND VETERINARY ISOLATES

N. MOHSENI[1], M. RAD[1], B. KHORRAMIAN TOOSI[1], A. R. MOKHTARI[2], R. YAHYARAEYAT[3] & T. ZAHRAIE SALEHI[3]

[1]Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; [2]Technical Assistant for Microbiology of the Sobhe Salamat Alborz Food and Drug Lab; [3]Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Summary

Mohseni, N., M. Rad, B. Khorramian Toosi, A. R. Mokhtari, R. Yahyaraeyat & T. Zahraie Salehi, 2021. Evaluation of MexAB-OprM efflux pump and determination of antimicrobial susceptibility in *Pseudomonas aeruginosa* human and veterinary isolates. *Bulg. J. Vet. Med.,* **24**, No 2, 200–207.

Pseudomonas aeruginosa could cause serious infections in hospitals and is highlighted as a source of financial problems in farms. The revelation of drug resistant, particularly multi-drug resistant (MDR) *P. aeruginosa* is important around the world. The efflux pump activity is identified as one of the most important intrinsic resistant mechanisms in *P. aeruginosa*. A total of 96 *P. aeruginosa* isolates from inpatient and animal sources were tested for antimicrobial susceptibility and subjected to multiplex PCR (mPCR) assay to detect MexAB-OprM efflux pump system. The human isolates have shown the highest resistance against cefazolin, ampicillin, nalidixic acid, trimethoprim/sulfamethoxazole, cephalothin, oxacillin (100%). All farm animal isolates were resistant to cefazolin, kanamycin, amoxicillin clavulanic acid, and cephalothin (100%). In both isolate groups, the presence of MexA was more common than that of MexB. MexAB-OprM was demonstrated as a valuable mechanism in *P. aeruginosa* antimicrobial resistant strains.

Key words: antimicrobial susceptibility, efflux pump, MexAB-OprM, multiplex PCR, *Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa, a Gram-negative rod shaped bacterium, an opportunistic pathogen for humans, animals, insects, plants and nematodes (Beinlich *et al.*, 2001; Rubin *et al.*, 2008). Infection in animals by *P. aeruginosa* can lead to financial problems in farm. Also *P. aeruginosa* is known as one of the most important bacteria that cause serious infections in hospitals (Beinlich *et al.,* 2001; Bhatt *et*

al., 2015). The revelation of drug resistant, particularly multi-drug resistant (MDR) among *P.aeruginosa* is a highlighted issue around the world, as this organism has shown high rates of resistance against biocides, detergents, dyes and disinfectants (Gibb *et al.*, 2002; Schweizer, 2003; El Zowalaty *et al.*, 2015; Potron *et al.*, 2015).

There are several mechanisms that make bacteria resistant to antimicrobial agents, including enzymatic inactivation, changing the permeability of outer membrane, and efflux pump activity (Lambert, 2002; Lister *et al.*, 2009; Chalhoub *et al.*, 2017). The most important intrinsic resistance mechanism in *P. aeruginosa* is related to synergy between reducing outer membrane permeability and efflux pump activity, named resistance nodulation division (RND) (Dean *et al.*, 2003). Four efflux pumps, MexAB-OprM (ABM), MexCD-OprJ (CDJ), MexEF-OprN (EFN), and MexXY-OprM (XY) were demonstrated in *P. aeruginosa* (Hocquet *et al.*, 2007). The RND pumps are composed of inner membrane transporters (i.e., MexB, MexD, MexF, and MexY) which seem to fusion a proton, an outer membrane component (i.e., OprM, OprJ, and OprN) helping forming channel, and a membrane fusion protein (i.e., MexA, MexC, MexE, and MexX) (Pearson *et al.,* 1999; Dean *et al.,* 2003; Schweizer, 2003). Previous surveys have shown that the MexAB-OprM and MexXY-OprM are more important in the natural resistance of *P. aeruginosa,* also the MexAB-OprM efflux system has a significant role for the virulence of this bacterium (Evans *et al.*, 1998; Masuda *et al.*, 2000; Llanes *et al.*, 2004; Sobel *et al.*, 2005).

The number of antimicrobial drug resistant *P. aeruginosa* strains are increasing and as efflux pump was determined as one of the most remarkable mechanisms in resistant strains, the current study was conducted to identify efflux pump genes and determine antimicrobial susceptibility among *P. aeruginosa* isolates from inpatients and farm animals.

MATERIALS AND METHODS

Isolation and identification

A total of 96 *P. aeruginosa* isolates including 50 isolates from two hospitals in Mashhad, and 46 isolates from bovine mastitis cases in Tehran and Mashhad, were collected. The isolates purification was done by culturing on differential and selective media like cetrimide agar. Then isolates were confirmed by biochemical tests as followed: oxidase/catalase, citrate utilization test, triple sugar iron agar (TSI), urease activity, and motility.

DNA extraction and verification of determined isolates by PCR assay

DNA was extracted from each isolate using boiling method (Ahmed & Dablool, 2017). All isolates were identified as *P. aeruginosa* by using 16S rRNA specific primers. The forward and reverse primer sequences were GGGGGATCTTCGG ACCTCA and TCCTTAGAGTGCCCA CCCG, respectively (Spilker *et al.*, 2004). The PCR reaction was carried out in 25 µL volume containing: 12.5 µL of PCR 2× MasterMix (Parstous, Iran) containing Taq DNA Polymerase, reaction buffer, dNTPs mixture, protein stabilizer, and the convenience for use was optimised by adding sediment for electrophoresis and 2× solution of loading dye, 3 µL of DNA template (150 ng/reaction), 7.5 µL nuclease-free water, 1 µL of forward primer and reverse primer. PCR cycles were as followed: initial denaturation at 95 °C for

2 min; followed by 25 cycles of denaturation at 94 °C for 20 s, annealing at 58 °C for 20 s, and extension at 72 °C for 40 s with a final extension for 1 min.

Antimicrobial susceptibility tests

The susceptibility of isolates against different classes of antibiotics was assessed using the disc diffusion method (CLSI, 2018). The susceptibility of all isolates were determined to gentamicin (10 μg), cefazolin (30 μg), ceftriaxone (30 μg), ampicilin (25 μg), nalidixic acid (30 μg), trimethoprim sulfamethoxazole (25 μg), cephalothin (30 μg), levofloxacin (5 μg), and tetracycline (30 μg) (Mast Co., UK). In addition, azetronam (30 μg), oxacillin (1 μg), and ciprofloxacin (5 μg) were used just for human isolates, while kanamycin (30 μg), enrofloxacin (5 μg) and amoxicillin/clavulanic acid (30 μg) used for animal isolates.

Evaluation of MexAB-OprM

The MexAB-OprM efflux pumps were detected using m-PCR. *P. aeruginosa* ATCC 27853 and ATCC 2027 strains and distilled water were used as positive and negative controls, respectively. The information of primer sequences is listed in Table 1. In this order, PCR reaction was run in 25 μL volumes including: 12.5 μL of PCR 2× MasterMix (Parstous, Iran) containing Taq DNA Polymerase, reaction buffer, dNTPs mixture, protein stabilizer, and the convenience for use was optimised by adding sediment for electrophoresis and 2× solution of loading dye, 3 μL of DNA template (150 ng/reaction), 7.5 μL nuclease-free water, 1 μL of each primer.

PCR programme was as followed: initial denaturation at 94 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 1 min with a final extension for 5 min.

RESULTS

Antimicrobial susceptibility tests

The highest resistance among human isolates (100%) was observed against cefazolin, ampicilin, nalidixic acid, trimethoprim sulfamethoxazole, cephalothin, oxacillin. Also, the second highest resistance (96%) was shown to tetracycline.

Among 46 mastitis isolates, the highest resistance was recorded to cefazolin, kanamycin, amoxicillin/clavulanic acid, cephalothin (100%) while the lowest resistance was exhibited to levofloxacin and gentamicin (0%). Information of isolates' susceptibility is listed in Table 2 and Table 3, respectively.

Antimicrobial susceptibility patterns

The antimicrobial susceptibility presented several different resistance patterns among all isolates (Table 4). The most frequent resistance pattern among human isolates was against cefazolin, ampicillin, nalidixic acid, trimethoprim sulfamethoxazole, tetra-

Table 1. Oligonucleotide primers

Primer name	Primer sequences*	Product (bp)
MexA	F: CTCGACCCGATCTACGTC R: GTCTTCACCTCGACACCC	503
MexB	F: TGTCGAAGTTTTTCATTGAG R: AAGGTCAC GGTGATGGT	280

*Arabestani *et al.* (2015).

Table 2. Percentage of resistant strains among human isolates (n=50)

	Behaviour of isolates		
	Resistant	Intermediate	Susceptible
Ampicillin	100	0	0
Oxacillin	100	0	0
Cephalothin	100	0	0
Ceftriaxone	9	26	65
Cefazolin	100	0	0
Tetracycline	96	0	4
Gentamicin	34	0	66
Nalidixic acid	100	0	0
Ciprofloxacin	20	4	76
Levofloxacin	32	4	64
Aztreonam	40	8	52
Trimethoprim/sulfa-methoxazole	100	0	0

Table 3. Percentage of resistant strains among bovine mastitis isolates (n=46)

	Behaviour of isolates		
	Resistant	Intermediate	Susceptible
Ampicillin	97.8	0	2.1
Amoxicillin/clavu-lanic acid	100	0	0
Cephalothin	100	0	0
Ceftriaxone	8.8	26	65.2
Cefazolin	100	0	0
Tetracycline	78.2	0	21
Gentamycin	0	0	100
Amikacin	100	0	0
Nalidixic acid	97.8	0	2.1
Enrofloxacin	6.5	60.8	32.6
Levofloxacin	0	0	100
Trimethoprim/sulfa-methoxazole	86.9	10.8	2.1

cycline, cephalothin and oxacillin (34%). Also, resistance pattern to cefazolin, amikacin, amoxicillin-clavulanic, ampicillin, trimethoprim sulfamethoxazole, tetracycline, and cephalothin was determined as the most frequent pattern among isolates from farm animals (60.8%).

Detection of MexAB-OprM

The m-PCR procedure revealed that frequency of MexA mRNA was higher than MexB in all isolates. Generally, MexA mRNA was detected in 35 out of 50 human isolates (70%), and MexB was positive in other 15 human isolates. Also, MexA mRNA was more prevalent than

Table 4. Antimicrobial resistance patterns for studied *P. aeruginosa* isolates

Resistance patterns	Number (%)
Human isolates (n=50)	
AMP, OXA, CEP, CFZ, TET, NAL, SXT	17 (34%)
AMP, OXA, CEP, CFT, CFZ, TET, GEN, NAL, CIP, LVX, ATM, SXT	9 (18%)
AMP, OXA, CEP, CFT,CFZ, TET, NAL, SXT	8 (16%)
AMP, OXA, CEP, CFT, CFZ, TET, NAL, ATM, SXT	6 (12%)
AMP, OXA, CEP, CFT, CFZ, TET, NAL, GEN, CIP, LVX, SXT	3 (6%)
AMP, OXA, CEP, CFT, CFZ, TET, GEN, NAL, LVX, ATM, SXT	2 (4%)
AMP, OXA, CEP, CFT, CFZ, TET, NAL, CIP, LVX, ATM, SXT	1 (2%)
AMP, OXA, CEP, CFT, CFZ, TET, GEN, NAL, SXT	1 (2%)
AMP, OXA, CEP, CFT, CFZ, TET, NAL, LVX, ATM, SXT	1 (2%)
AMP, OXA, CEP, CFZ, TET, GEN, NAL, SXT	1 (2%)
AMP, OXA, CEP, CFZ, TET, GEN, NAL, ATM, SXT	1 (2%)
Bovine mastitis isolates (n=40)	
AMP, AUG, CEP, CFZ, TET, AMK, SXT	28 (60.8%)
AMP, AUG, CEP, CFZ, AMK, SXT	7 (15.2%)
AMP, AUG, CEP, CFZ, AMK	3 (6.5%)
AMP AUG, CEP, CFZ, TET, AMK	2 (4.3%)
AMP, AUG, CEP, CFZ, TET, AMK, ENR, SXT	2 (4.3%)
CEP, CFZ, TET, AMK, NAL, SXT	1 (2.1%)
AMP, AUG, CEP, CFT, CFZ, TET, AMK, SXT	1 (2.1%)
AMP, AUG, CEP, CFT, CFZ, TET, AMK, SXT	1 (2.1%)
AMP, AUG, CEP, CFT, CFZ, TET, AMK, NAL, ENR, SXT	1 (2.1%)

AMP, Ampicillin; AUG, Amoxicillin/Clavulanic; ; OXA, Oxacillin; CEP, Cephalothin; CFT, Ceftriaxone; CFZ, Cefazolin; TET, Tetracycline; GEN, Gentamicin; AMK, Amikacin; NAL, Nalidixic acid; CIP, Ciprofloxacin; ENR, Enrofloxacin; LVX, Levofloxacin; ATM, Aztreonam; SXT, Trimethoprim/Sulfamethoxazole.

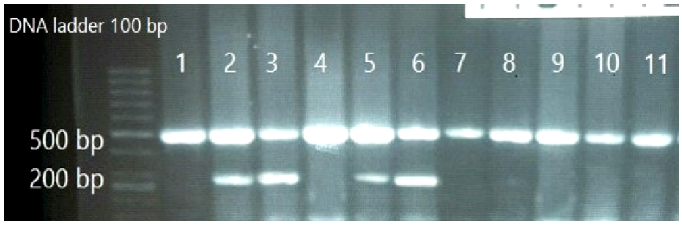

Fig. 1. Multiplex PCR on gel electrophoresis. Lanes 2, 3, 5, 6: human isolates from hospitals with MexA and MexB; lanes 1, 7–11: human isolates from hospitals with MexA.

MexB among mastitis isolates, 40 (86.9%) and 11 (23.9%), respectively (Fig. 1).

DISCUSSION

P. aeruginosa is an opportunistic pathogen known as a remarkable organism in hospitals, as well as one of the most important causes of mastitis at farms (Mekic *et al.*, 2011; Bhatt *et al.,* 2015). On the other hand, the development of antimicrobial resistance, particularly multidrug-resistance (MDR), extended drug resistance (XDR), and pandrug resistance

(PDR), is a serious concern at a global scale. The high prevalence of MexA mRNA (78.1%) and MexB mRNA (21.8%) in our study had confirmed results from previously surveys (Beinlich *et al.,* 2001; Hocquet *et al.,* 2007; Al-Grawi, 2012; Khosravi & Mohammadian, 2016). Also, our findings detecting MexAB-OprM mRNA in all isolates and recording high rate of MDR strains, demonstrated that MexAB-OprM mRNA had a significant role in MDR strains, in line with a previous study by Arabestani *et al.* (2015), and another study by Goli *et al.* (2016) which had reported overexpression of MexB mRNA (76%) among of MDR strains. The existence of MexAB-OprM at the RNA level is a prerequisite for expression of MDR pumps and therefore, resistance against antibiotics.

According to antimicrobial susceptibility tests, cefazolin, ampicillin, tetracycline, cephalothin and trimethoprim sulfamethoxazole, have shown the lowest activity against all isolates. In addition, all human isolates were resistant to oxacillin and nalidixic acid, as well as all mastitis isolates were resistant to kanamycin and amoxicillin/clavulanic acid. However, gentamicin and ciprofloxacin had exhibited most commonly activity (66–100%) to all isolates, respectively. This finding corroborated with other survey results in Iran, which had reported high rate of susceptibility to ciprofloxacin (Mokhtari *et al.*, 2016; Ranji & Rahbar Takrami, 2017) but disagreed with other earlier studies, which had determined lower rate of susceptibly to ciprofloxacin (Japoni *et al.,* 2006; Zhang *et al.*, 2014; Arabestani *et al.,* 2015; Goli *et al.,* 2016; Khosravi & Mohammadian, 2016). There are several possible explanations for this conflicting outcome, such as geographical differences, variability of conventional antibiotics in the regions.

According to resistance phenotype against at least one of the aminoglycosides, penicillins, cephalosporins, and antimetabolites antibiotic families, all isolates are known as MDR. The rate of MDR strains in this study was higher compared to previously findings (66% – 76%) (Japoni *et al.,* 2006; Rubin *et al.,* 2008; Goli *et al.,* 2016; Khosravi & Mohammadian, 2016). The apparent lack of correlation can be justified by increased use of antibiotics, sampling, and spread of resistance genes with time. The high activity of gentamicin and levofloxacin (100%) against mastitis isolates must be considered when using these antibiotics for treating mastitis in farms.

CONCLUSION

According to the results, MexAB-OprM might have a remarkable role in turning *P. aeruginosa* strains into resistant strains. However, large-scale studies are required to evaluate the relation between MexAB-OprM and resistant strains. Also, increased prevalence of MDR strains is an important threat in hospitals all around the world requiring more essential surveillance on prescribing these antibiotics in veterinary practice.

REFERENCES

Ahmed, O. B. & A. S. Dablool, 2017. Quality Improvement of the DNA extracted by boiling method in Gram negative bacteria. *International Journal of Bioassays,* **6**, 5347–5349.

Al-Grawi, I. G. A., 2012. Occurrence of MexAB-OprM efflux pump operon on septicemic *Pseudomonas aeruginosa* chro-

mosome. *Iraqi Academic Scientific Journal,* **11**, 97–102.

Arabestani, M. R., M. Rajabpour, R. Yousefi Mashouf, M. Y. Alikhani & S. M. Mousavi, 2015. Expression of efflux pump MexAB-OprM and OprD of *Pseudomonas aeruginosa* strains isolated from clinical samples using qRT-PCR. *Archives of Iranian Medicine,* **18**, 102–108.

Beinlich, K. L., R. Chuanchuen & H. P. Schweizer, 2001. Contribution of multidrug efflux pumps to multiple antibiotic resistance in veterinary clinical isolates of *Pseudomonas aeruginosa*. *FEMS Microbiology Letters,* **198**, 129–134.

Bhatt, P., K. R. Rathi, S. Hazra, A. Sharma & V. Shete, 2015. Prevalence of multidrug resistant *Pseudomonas aeruginosa* infection in burn patients at a tertiary care centre. *Indian Journal of Burns,* **23**, 56.

Chalhoub, H., D. Pletzer, H. Weingart, Y. Braun, M. M. Tunney, J. S. Elborn, H. Rodriguez-Villalobos, P. Plésiat, B. C. Kahl & O. Denis, 2017. Mechanisms of intrinsic resistance and acquired susceptibility of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients to temocillin, a revived antibiotic. *Scientific Reports,* **7**, 40208.

CLSI, 2018. Performance Standards for Antimicrobial Susceptibility Testing. 28th edn, CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Dean, C. R., M. A. Visalli, S. J. Projan, P.-E. Sum & P. A. Bradford, 2003. Efflux-mediated resistance to tigecycline (GAR-936) in *Pseudomonas aeruginosa* PAO1. *Antimicrobial Agents and Chemotherapy,* **47**, 972–978.

El Zowalaty, M. E., A. A. Al Thani, T. J. Webster, A. E. El Zowalaty, H. P. Schweizer, G. K. Nasrallah, H. E. Marei & H. M. Ashour, 2015. *Pseudomonas aeruginosa:* Arsenal of resistance mechanisms, decades of changing resistance profiles, and future antimicrobial therapies. *Future Microbiology,* **10**, 1683–1706.

Evans, K., L. Passador, R. Srikumar, E. Tsang, J. Nezezon & K. Poole, 1998. Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. *Journal of Bacteriology,* **180**, 5443–5447.

Gibb, A. P., C. Tribuddharat, R. A. Moore, T. J. Louie, W. Krulicki, D. M. Livermore, M.-F. I. Palepou & N. Woodford, 2002. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new blaIMP allele, blaIMP-7. *Antimicrobial Agents and Chemotherapy,* **46**, 255–258.

Goli, H. R., M. R. Nahaei, M. A. Rezaee, A. Hasani, H. S. Kafil, M. Aghazadeh & V. Sheikhalizadeh, 2016. Contribution of mexAB-oprM and mexXY (-oprA) efflux operons in antibiotic resistance of clinical *Pseudomonas aeruginosa* isolates in Tabriz, Iran. *Infection, Genetics and Evolution,* **45**, 75–82.

Hocquet, D., M. Roussel-Delvallez, J.-D. Cavallo & P. Plésiat, 2007. MexAB-OprM-and MexXY-overproducing mutants are very prevalent among clinical strains of *Pseudomonas aeruginosa* with reduced susceptibility to ticarcillin. *Antimicrobial agents and chemotherapy,* **51**, 1582–1583.

Japoni, A., A. Alborzi, M. Kalani, J. Nasiri, M. Hayati & S. Farshad, 2006. Susceptibility patterns and cross-resistance of antibiotics against *Pseudomonas aeruginosa* isolated from burn patients in the South of Iran. *Burns,* **32**, 343–347.

Khosravi, A. D. & A. Mohammadian, 2016. Efflux MexAB-mediated resistance in multidrug and pan-drug resistant strains of *Pseudomonas aeruginosa* isolated from patients with burn and wound infections. *Jundishapur Journal of Natural Pharmaceutical Products,* **11**, e25352.

Lambert, P., 2002. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *Journal of the Royal Society of Medicine,* **95**, 22.

Lister, P. D., D. J. Wolter & N. D. Hanson, 2009. Antibacterial-resistant P*seudomonas aeruginosa:* Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews,* **22**, 582-610.

Llanes, C., D. Hocquet, C. Vogne, D. Benali-Baitich, C. Neuwirth & P. Plésiat, 2004. Clinical strains of *Pseudomonas aeruginosa* overproducing MexAB-OprM and MexXY efflux pumps simultaneously. *Antimicrobial Agents and Chemotherapy,* **48**, 1797–1802.

Masuda, N., E. Sakagawa, S. Ohya, N. Gotoh, H. Tsujimoto & T. Nishino, 2000. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy,* **44**, 3322–3327.

Mekic, S., K. Matanovic & B. Seol, 2011. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates from dogs with otitis externa. *The Veterinary Record,* **169**, 125–125.

Mokhtari, A. R., T. Zahraee Salehi, K. Amini & F. Shahcheraghi, 2016. Isolation *Pseudomonas aeruginosa* bacteria and genes integron class I of subclinical mastitis in dairy cows in Tehran. *Veterinary Researches & Biological Products,* **29**, 37–44.

Pearson, J. P., C. Van Delden & B. H. Iglewski, 1999. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *Journal of bacteriology,* **181**, 1203–1210.

Potron, A., L. Poirel & P. Nordmann, 2015. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology. *International Journal of Antimicrobial Agents,* **45**, 568–585.

Ranji, N. & S. Rahbar Takrami, 2017. Role of mexZ gene in ciprofloxacin resistance in *Pseudomonas aeruginosa* isolates in Guilan province. *Urmia Medical Journal,* **27**, 902–913.

Rubin, J., R. Walker, K. Blickenstaff, S. Bodeis-Jones & S. Zhao, 2008. Antimicrobial resistance and genetic characterization of fluoroquinolone resistance of *Pseudomonas aeruginosa* isolated from canine infections. *Veterinary Microbiology,* **131**, 164–172.

Schweizer, H. P., 2003. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. *Genetics and Molecular Research,* **2**, 48–62.

Sobel, M. L., D. Hocquet, L. Cao, P. Plesiat & K. Poole, 2005. Mutations in PA3574 (nalD) lead to increased MexAB-OprM expression and multidrug resistance in laboratory and clinical isolates of *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy,* **49**, 1782–1786.

Spilker, T., T. Coenye, P. Vandamme & J. J. Lipuma, 2004. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. *Journal of Clinical Microbiology,* **42**, 2074-2079.

Zhang, X., S. Niu & L. Zhang, 2014. Antimicrobial susceptibilities and clinical characterization of *Pseudomonas aeruginosa* isolates from urinary tract infections. *Urologia Internationalis,* **93**, 464–469.

Paper received 08.05.2019; accepted for publication 21.09.2019

Correspondence:

M. Rad
Department of Pathobiology,
School of Veterinary Medicine,
Ferdowsi University of Mashhad,
Mashhad, Iran
e-mail: rad@um.ac.ir