ORIGINAL ARTICLE

Investigation of Antibiotic Resistance Pattern of Non-Fermenting Gram-Negative Bacteria Isolated from patients in a referral hospital, Shiraz, Southwest Iran

MOHAMMAD JAFARINODOUSHAN[1], MOHAMMAD MOTAMEDIFAR[2], NAVIDOMIDIFAR[3], MAHDI RAHMANIAN[1,4]
[1]*Student Research Committee, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.*
[2]*HIV/AIDS Research Center, Institute of Health AND Department of Bacteriology & Virology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.*
[3]*Department of Pathology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.*
[4]*MPH Department, Shiraz University of Medical Sciences, Shiraz, Iran.*
Correspondence to Dr. Mohammad Motamedifar, Ph.D. ,Email: motamedm@sums.ac.ir, Tel/Fax:09173147718

ABSTRACT

Aim: Antimicrobial resistance isa global concern. Bacterial resistance to the available antibiotics can lead to increased morbidity and mortality. In this study, we investigated the frequency and antibiotic resistance pattern ofnon-fermenting gram-negative bacteria *Acinetobacter baumannii* and *Pseudomonas aeroginosa.*
Methods: This is a cross-sectional study was conducted in a referral hospital, through a questionnaire that contained patients' age, gender, site of the body where sample was taken, severity of microbial growth in culture, type of organism that was found in the culture, and the antibiogram result.
Results: A total of 210 patients (7 adults and 203 neonates) were included in this study. The most frequent antibiotics that *A. baumannii* was resistant to were amikacin (89.4%), ceftazidime (86.5%), gentamicin (70.9%), imipenem (70.9), cefepime (67.4%), ciprofloxacin (60.3%), cotrimoxazole (36.2%), tetracycline (9.9%), and cefotaxime (5.7%). The most frequent antibiotics that *P. aeroginosa* was resistant to were amikacin (31.9%), gentamicin (31.9%), ceftazidime (29%), cefepime (24.6%), ciprofloxacin (11.6%), and imipenem (10.1%), respectively.
Conclusion: The lowest resistance rates of *A. baumanni* isolates to the studied antibiotics were related to tetracycline and cefotaxime, and the lowest resistance rates of *P.aeroginosa* isolates were related to imipenem and ciprofloxacin.
Keywords: Gram-Negative Bacterial Infections, Acinetobacter, Pseudomonas, Neonate, Iran

INTRODUCTION

Antibiotics allow the body's immune system to eliminate pathogenic micro-organisms through their cytostatic or cytotoxic effects. These effects are through some mechanisms, such as inhibiting the synthesis of the bacterial cell, proteins,deoxyribonucleic acid, ribonucleic acid, and disorganizing micro-organisms' membrane[1].

Since the introduction of the first effective antibiotic, the phenomenon of bacterial resistance to antibiotics became noticeable and soon it became one of the most important concerns in the management of bacterial infections[2].

Antimicrobial resistance transpires through different mechanisms including intrinsic resistance, acquired resistance, genetic change, and DNA transfer [3],and even the natural selection process is considered to play a role in this phenomenon[4].

The most antibiotic resistance rate is reported in the regions of the world with the most use of antibiotics[2]. Prescribing too many unnecessary antibiotics by physicians[5] as well as their usage in animal-husbandry and agricultural industry, living in poor hygienic areas, increased migration to urban areas, and insufficient sewerage disposal system are considered as possible risk factors for developing bacterial resistance to antibiotics[6].

Bacterial resistance to the available antibiotics can cause increased morbidity and mortality. The emergence of multidrug resistance is catastrophic for humans, animals as well as environment[2]. Antibiotic resistance has causedover 23000 deaths per year and over 20 billion dollars additional medical expenses in the United States[3].

Antibiotic resistance amongst the gram-negative bacteria is now a global concern; furthermore, the growing number of multi-drug resistance species are becomingtoo difficult to treat. Amongst them, non-fermenters can develop into multidrug resistance, a major healthcare concern. These pathogens are opportunistic infections, and the most prevalent species are *Pseudomonas*, *Acinetobacter*, *Stenotrophomonasmaltophilia*, and *Burkholderiacepacia* complex[7].

In order to find out the antibiotic resistance pattern of Acinetobacter *baumannii* (*A. baumannii*) and *Pseudomonas aeroginosa* (*P. aeroginosa*) in our setting, we investigated the frequency and antibiotic resistance pattern in different samples, obtained from patients in a referral hospital.

MATERIALS AND METHODS

Study design, setting, and participants: This cross-sectional study was performedbetween April 2017 to December 2018. Out of 11,596 cultures taken in Zeinabieh Hospital, 210 were positive samples, and the rest were either negative or did not have antibiogram. Zeinabieh is a referral hospital, affiliated with Shiraz University of Medical Sciences (SUMS) and is specializing in obstetrics and gynecology fields. SUMS is located in Shiraz-the capital of Fars province- and is among the top five medical sciences universities in Iran. Our participants were either newborns

or their mothers.The local Ethics Committee of the membrane of SUMS approved this study (approval code: IR.SUMS.MED.REC.1397.450).

Data collection and variables: All data were collected by searching the registration program that includes patients' laboratory data during their hospitalization. Only patients who had positive cultures with an antibiotic susceptibility tests were included in this study. We filled a questionnaire for each patient, containing their unit number, date of hospitalization, age, gender, site of body where sample was taken, severity of microbial growth in culture, type of organism that was found in the culture, and the antibiogram result.

Data preparation and statistical analysis: Admission date of patients was categorized into two subgroups of those who were either admitted in 2017 or 2018. The site of the body where samples were taken was categorized into nine subgroups including blood, endotracheal tube (ETT), cerebrospinal fluid (CSF), eye, throat, intravenous line, urine, wound, and other sites (nose, anus, venous catheter, and skin).Sterile samples were immediately transported to the microbiology laboratory and cultured on blood agar, chocolate agar, and Mac Conkey's agar, incubated at 37°C for 24 hours. Identification of bacteria was completed according to standard microbiological procedures[8].

Antibiotic susceptibility testing was done by disc diffusion method, according to the CLSI standards[9], using amikacin, ceftazidime, ciprofloxacin, cefepime, gentamicin, and Imipenem for both of the studied bacteria and trimethoprim sulfamethoxazol (cotrimoxazole), tetracyclin, and cefotaxime just for *A.baumannii*. We used frequency (percent of frequency) and mean ± standard deviation (SD) for descriptive analysis and Pearson Chi-Square test for bivariate analysis. SPSS version 25 was used for analysis.

RESULTS

Of all non-fermenting gram-negative bacteria only *A.baumannii* and *P.aeroginosa* were detected.Of all the positive samples, 203(96.7%) were related to neonates and only 7(3.3%) belonged to adult patients. One-hundred-eighteen (58.1%) of neonates were male, and all the adults were female. The mean age of adult patients was 47.14±18.47. Amongst the adult patients, 2(28.2%), 1(14.3%), 1(14.3%), and 3(42.9%) had positive cultures from urine, ETT, wound, and other site samples, respectively. Amongst the neonates, the most frequent sites of infection were throat with 36 (17.7%), ETT with 19(9.4%) and eye 14 (6.9%) (Table1).

Of the 7 adults, 2 were positive for *A. baumannii* (1 from ETT sample and 1 from wound sample) and 5 were positive for *P. aeroginosa* (2 from a urine sample and 3 from other sites samples). Amongst neonates,139(68.5%) and 64(31.5%) were positive for *A. baumannii* and *P. aeroginosa*, respectively (Table 2).

Of all the adult patients, 6(85.7%) had a heavy growth of bacteria in their culture. The information for one adult patient was missing. Twenty-five (12.3%), 22 (10.8%), and 119 (58.6%) of neonate patients had a mild, moderate, and heavy growth of bacteria in culture. The information in this regard for 37 neonate patients was missing.

Of all *A.baumannii* positive samples 126 (89.4%), 122(86.5%), 100(70.9%), 100(70.9%), 95(67.4%), 85(60.3%), 51(36.2%), 14(9.9%), and 8(5.7%) were resistant to amikacin, ceftazidime, gentamicin, imipenem, cefepime, ciprofloxacin, cotrimoxazole, tetracycline, and cefotaxime, respectively. Pearson Chi-Square test showed that susceptibility of*A.baumannii* to cefepime (P<0.001), gentamicin (P=0.026), and imipenem (P=0.009) decreased, but to tetracyclin (P<0.001) increasedin2018 (Figure 1).

Of all *P.aeroginosa* positive samples, 22(31.9%), 22(31.9%), 20(29%), 17 (24.6%), 8 (11.6%), and 7(10.1%) were resistant to amikacin, gentamicin, ceftazidime, cefepime, ciprofloxacin, and imipenem, respectively. Pearson Chi-Square test showed that there was no statistical relevance according to the studied antimicrobial susceptibility to *P. aeroginosa* during the study period (Fig. 2).

Table 1: Sites of infection in neonates in both genders

	Throat	**ETT**	**Eye**	**Blood**	**CSF**	**Urine**	**Other sites**	**Total**
Male (%))	22 (18.6)	13 (11)	7 (5.9)	8(6.8)	1 (0.8)	0 (0)	67 (56.8)	118 (100)
Female(%)	14 (16.5)	6 (7.1)	7 (8.2)	5(5.9)	1 (1.2)	1 (1.2)	51 (60)	85 (100)

Table 2. Frequency of bacteria culture based on sites of infection

	Throat	**ETT**	**Eye**	**Blood**	**CSF**	**Urine**	**Other sites**	**Total**
A.baumannii (F(%))	20 (14.2)	9 (6.4)	5 (3.5)	10(7.1)	2 (1.4)	1 (0.7)	93 (66)	141 (100)
P.aeroginosa (F(%))	16 (23.2)	11(15.9)	9 (13)	3 (4.3)	0 (0)	2 (2.9)	28 (40.6)	69 (100)

Figuire 1. Pattern of antibiotic resistance to *Acinetobacter baumannii*

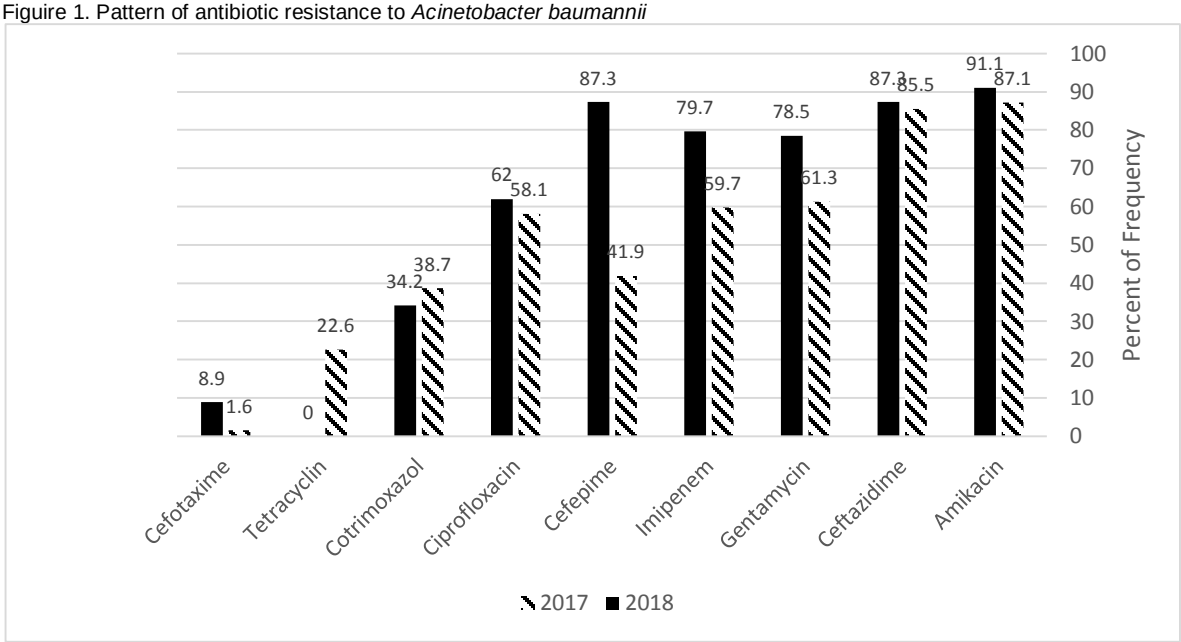
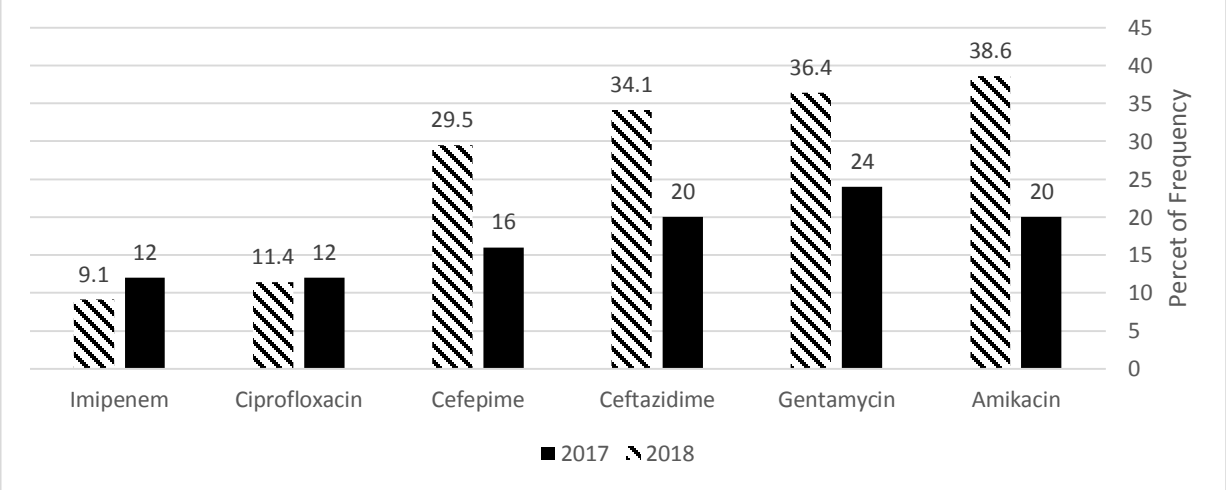


Figure 2. Pattern of antibiotic resistance of *Pseudomonas aeroginosa*



DISCUSSION

Amongst our adult patients, the most infected site was urinary tract, while throat was the most frequent site between neonates. These results were almost in concordance with the studies done by Kamat et al. in India [10] and by Davoudi et al. in Iran [11].Urinary tract and respiratory tract are amongst the most common sites involved in nosocomial infections.

The most frequent antibiotic that *A.baumannii*was resistant to, was amikacin and the least frequent was cefotaxime. A study by Villalo´n et al. in Spain showed that all isolates of *A.baumanii* were fully resistant to ticarcillin, piperacillin, piperacillin-tazobactam, aztreonam, cefotaxime, ceftazidime, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole. The rate of resistance to other antibiotics was 98.3% for cefepime, 74.4% for imipenem, and 89.8% for amikacin[2] A study by Ruoming Tan et al. in China showed that the resistance rate of hospital-wide *A.baumannii* to amikacin, cotrimoxazole, ciprofloxacin, ceftazidime, cefepime, and imipenem was 51.4%, 62.4%, 62.6%, 58.2%, 58.6%, and 44.2% respectively[13]. A study by Maraki et al. in Greeceshowed that the resistance rate of *A.baumannii* isolates for cefotaxime, ceftazidime, cefepime, amikacin, gentamycin, tetracycline, and ciprofloxacin was 83.2%, 79.6%, 82.5%, 67.2%, 70.8%, 80.3%, and 85.5%, respectively[14]. In another study by Karlowsky et al. in the USA<10% of *A.baumannii* were resistant to imipenem, from 20 to 30% of isolates were resistant to amikacin, and more than 40%

were resistant to ceftazidime, ciprofloxacin, and gentamycin[15]. A study by Lăzureanu et al. in Romania revealed that resistance rate of *A.baumannii* forceftazidime and imipenem was 94.6% each, for cefepime 89.2%, and for cefotaxime 91.9%[16]. A study by Fitzpatrick et al. in the USA showed that the resistance rate of *A.baumannii* isolates for imipenem, cefepime, ciprofloxacin, and amikacin was 75%, 80%, 82%, and 61%, respectively[17]. A meta-analysis by Pourhajibaghe et al. in Iran showed that 55% of *A.baumannii*were resistant to imipenem[18]. Ezzat et al. in Egypt showed that all strains of *A.baumannii* were resistant to ceftazidime, cotrimoxazole, ceftriaxone, and cefepime. The resistance rate of *A.baumannii* to imipenem, gentamycin, and amikacin was 33.33%, 55.56%, and 55.56%, respectively[19]. A study done by Asadian et al. in Iran showed that the resistance rate of *A.baumannii*to imipenem, cotrimoxazole, cefotaxime, tetracycline, amikacin, ciprofloxacin, ceftazidime, gentamycin, and cefepime was 79.7%, 89.8%, 97.4%, 74.6%, 81%, 96.2%, 97.4%, 92.4%, and 87.3%, respectively[20]. A study by malekzadegan et al. in Iran showed that all isolates of *A.baumannii* were resistant to imipenem. It also showed that 83.3% of *A.baumannii* isolates were resistant to ceftazidime[21]. Considering all these studies including ours, none of the mentioned antibiotics seems to be appropriate as gold standard treatment for *A.baumannii*. It used to be believed that carbapenems were the mainstay against multi-drug resistant *A.baumanii*[22] while in many settings worldwide including ours due to high resistance rate, is no longer recommended. Surprisingly the resistance rates of *A.baumanii* isolates to tetracyclin and cefotaxime were low in this study, and the reason might be due toits lower rate of usage in our setting. For being able to recommend these two antibiotics as drugs of choice against *A.baumanii*more investigation is warranted.

The most frequent antibiotic that *P.aeroginosa* was resistant to, was amikacin (31.9%) and the least resistance rate belonged to imipenem. In a study in Spain by Villalo´n et al. the resistance rate of hospital-wide *P.aeroginosa* was 23.9%, 31.5%, 26.3%, 25.7%, and 33.8% for amikacin, ciprofloxacin, ceftazidime, cefepime, and imipenem, respectively[12]. In another study by Fatima et al. in Pakistan showed that the resistance rate of *P.aeroginosa*to imipenem, amikacin, and cefepime were 24%, 35%, and 40%, respectively[23]. The resistance rate of *P.aeroginosa*to ceftazidime, cefepime, amikacin, gentamycin, and ciprofloxacin was 28.2%, 28.5%, 18.8%, 24.8%, and 32.2%, respectively as reported by Maraki et al. [14]. A study by Karlowsky et al. in the USA showed that <10% of *P.aeroginosa* isolates were resistant to amikacin, from 10 to 20% resistant to cefepime, ceftazidime, and imipenem, and from 20 to 30% of isolates were resistant to ciprofloxacin and gentamicin[15]. A study by Anyim et al. in Nygeria revealed that the resistance rate of *P.aeroginosa* to ceftazidime, ciprofloxacin, and gentamicin was 14.3%, 0.00%, and 71.4% respectively[24]. A study by Franse´n et al. in Sweden revealed that the resistance rate of P.aroginosato imipenem and ceftazidime was 26% and 15%, respectively[25]. Ezzat et al. reported that all *P.aeroginosa* isolates were resistant to Co-trimoxazole, ceftriaxone, and cefepime. The resistant rate of *P.aeroginosa* to ceftazidime, imipenem, gentamycin, and amikacin were 77.78%, 0%, 66.67%, and 66.67%, respectively[19]. A study by Pokharel et al. in Nepal showed that 63% *P.aeroginosa* were resistant to ceftazidime, 56.5% to cefotaxime, 37.4% to imipenem, 39.1% to ciprofloxacin, 42.2% to gentamycin, 26% to amikacin, and 80.4% to cotrimoxazole[26]. A study by Zare et al. in Iran showed that all *P.aeroginosa* isolates were resistant to cotrimoxazole and ceftriaxone[27]. It seems that *P. aeroginosa* isolates worldwide are still susceptible to imipenem and ciprofloxacin, unlike cefepime, ceftazidime, gentamycin, amikacin, and cotrimoxazole.

The major limitation of our study was that many of the hospitalized patients with positive cultures did not have any rerecorded antibiogram; hence, we had to exclude them from our study.

CONCLUSION

All in all, we found that amikacin is no longer suitable to treat *A.baumannii* and *P.aeroginosa*related infections in our setting. We also found that the lowest resistance rate of *A.baumannii*isolates to the studied antibiotics was related to tetracycline and cefotaxime and the lowest resistance rate of *P.aeroginosa* isolates was related to imipenem and ciprofloxacin. We recommend further studies with larger sample size in different settings and with various antibiotics in order to obtain a better understanding of *A.baumannii* and *P.aeroginosa* antibiotic susceptibility pattern.

Acknowledgment: This article was extracted from the doctor of medicine thesis written by Mohammad JafariNodoushan supervised by Dr. M. Motamedfar and Dr. N. Omidifar.The authors wish to thank Mr. H. Argasi at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for his invaluable assistance in editing this manuscript.

Funding: This study was funded by the Vice-Chancellor for Research of Shiraz University of Medical Sciences (grant number: 1396-01-01-15314).

REFERENCES

1. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature medicine 2004;10(12 Suppl):122-9.
2. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417-33.
3. Habboush Y, Guzman N. Antibiotic Resistance. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan.
4. Levy SB. From tragedy the antibiotic age is born. In:The Antibiotic Paradox. Springer, Boston, MA, 1992; pp 1-12.
5. Singer AC, Shaw H, Rhodes V, Hart A. Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. Front Microbiol 2016;7:1728.
6. Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health.Clinical microbiology reviews 2011;24:718-33.
7. McGowan Jr JE. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. The American journal of medicine 2006;119(6 Suppl 1):29-36.
8. Koneman E, Allen S, Janda W, Schreckenberger R, Winn W. Introduction to microbiology. In: Koneman EW, Alien SD, Janda WM, Schreckenberger RC, Winn W, editors. Part II, Guidelines for collection, transport, processing, analysis, and reporting of cultures from specific specimen sources, Color

Atlas and Textbook of Diagnostic Microbiology. 5th ed. Philadelphia: Lippincott, 1997; pp 121–70.
9. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, et al. CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests. J Clin Microbiol 2018;56:e01934-17.
10. Kamat U, Ferreira A, Savio R, Motghare D. Antimicrobial resistance among nosocomial isolates in a teaching hospital in goa. Indian J Community Med 2008;33:89-92.
11. Davoudi AR, Najafi N, Hoseini Shirazi M, Ahangarkani F. Frequency of bacterial agents isolated from patients with nosocomial infection in teaching hospitals of Mazandaran University of Medical Sciences in 2012. Caspian J Intern Med 2014;5:227-31.
12. Villalon P, Valdezate S, Medina-Pascual MJ, Rubio V, Vindel A, Saez-Nieto JA. Clonal diversity of nosocomial epidemic Acinetobacter baumannii strains isolated in Spain. J Clin Microbiol 2011;49:875-82.
13. Tan R, Liu J, Li M, Huang J, Sun J, Qu H. Epidemiology and antimicrobial resistance among commonly encountered bacteria associated with infections and colonization in intensive care units in a university-affiliated hospital in Shanghai.J Microbiol Immunol Infect 2014;47:87-94.
14. Maraki S, Mavros MN, Kofteridis DP, Samonis G, Falagas ME. Epidemiology and antimicrobial sensitivities of 536 multi-drug-resistant gram-negative bacilli isolated from patients treated on surgical wards. Surg Infect (Larchmt) 2012;13:326-31.
15. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of Pseudomonas aeruginosa and Acinetobacter baumannii from hospitalized patients in the United States, 1998 to 2001. Antimicrob Agents Chemother 2003;47:1681-8.
16. Lazureanu V, Porosnicu M, Gandac C, Moisil T, Baditoiu L, Laza R, et al. Infection with Acinetobacter baumannii in an intensive care unit in the Western part of Romania. BMC Infect Dis 2016;16 Suppl 1:95.
17. Fitzpatrick MA, Ozer E, Bolon MK, Hauser AR. Influence of ACB complex genospecies on clinical outcomes in a U.S. hospital with high rates of multidrug resistance.The Journal of Infection 2015;70:144-52.
18. Pourhajibagher M, Hashemi FB, Pourakbari B, Aziemzadeh M, Bahador A. Antimicrobial Resistance of Acinetobacter baumannii to Imipenem in Iran: A Systematic Review and Meta-Analysis. Open Microbiol J 2016;10:32-42.
19. Abd-Elmonsef MME, Elsharawy D, Abd-Elsalam AS. Mechanical ventilator as a major cause of infection and drug resistance in intensive care unit. Environ Sci Pollut Res Int 2018;25:30787-92.
20. Asadian M, Azimi L, Alinejad F, Ostadi Y, Lari AR. Molecular Characterization of Acinetobacter baumannii Isolated from Ventilator-Associated Pneumonia and Burn Wound Colonization by Random Amplified Polymorphic DNA Polymerase Chain Reaction and the Relationship between Antibiotic Susceptibility and Biofilm Production. Adv Biomed Res2019;8:58.
21. Malekzadegan Y, Abdi A, Heidari H, Moradi M, Rastegar E, Sedigh Ebrahim-Saraie H. In vitro activities of colistin, imipenem and ceftazidime against drug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii isolates in the south of Iran. BMC Res Notes 2019;12:301.
22. Kempf M, Rolain JM. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents 2012;39:105-14.
23. Fatima A, Naqvi SB, Khaliq SA, Perveen S, Jabeen S. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infections. Springerplus2012;1:70.
24. Anyim MC, Meka AO, Chukwu JN, Nwafor CC, Oshi DC, Madichie NO, et al. Secondary bacterial isolates from previously untreated Buruli ulcer lesions and their antibiotic susceptibility patterns in Southern Nigeria. Rev Soc Bras Med Trop 2016;49:746-51.
25. Fransen J, Huss FR, Nilsson LE, Rydell U, Sjoberg F, Hanberger H. Surveillance of antibiotic susceptibility in a Swedish Burn Center 1994-2012. Burns 2016;42:1295-303.
26. Pokharel K, Dawadi BR, Bhatt CP, Gupte S. Prevalence of Pseudomonas Aeruginosa and its Antibiotic Sensitivity Pattern. J Nepal Health Res Counc 2019;17:109-13.
27. Zare M, Torbati PM, Asadi-Amoli F, Talebnejad M, Parvizi M, Nasiri Z, et al. Microbiological Profile of Corneal Ulcers at a Tertiary Referral Center. Med Hypothesis Discov Innov Ophthalmol 2019;8:16-21.