Prevalence of colistin and tigecycline resistance in *Acinetobacter baumannii* clinical isolates from 2 hospitals in Riyadh Region over a 2-year period

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ABSTRACT

الأهداف: وصف معدل مقاومة عصويّات الأسنيتوبكتر تجاه المضادين كوليستين وتيجاسيكلين بين عينات الأسينيتوباكتر السريرية باثنين من مستشفيات منطقة الرياض الكبرى خلال فترة عامين متتاليين.

الطريقة: أجريت دراسة لسجلات مختبرات الأحياء الدقيقة بمستشفى الملك فيصل التخصصي ومركز الأبحاث وبمدينة الأمير سلطان العسكرية الطبية، الرياض، المملكة العربية السعودية للفترة الزمنية الممتدة من يناير 2010م إلى ديسمبر 2011م للتعرّف على العينات السريرية المستخلص منها جرثومة الأسينيتوباكتر بومانياي المقاومة للمضادين كوليستين وتيجاسيكلين خلال تلك الفترة.

النتائج: نتج عن البحث في السجلات المذكورة أعلاه مجموع 1307 من العينات السريرية التي أُستخلص منها بكتريا الأسينيتوباكتر والتي بلغت نسبة مقاومتها إجمالاً لمضادي تيجاسيكلين وكوليستين 9.7% و 1.8% من مجموع العينات على التوالي. هذا ولوحظ زيادة نسبة المقاومة للتيجاسيكلين بين عينات الأسينيتوباكتر من مستشفى الملك فيصل التخصصي من 10.4% في العام 2010م إلى 20.5% في العام التالي، بينما ارتفعت نسبة المقاومة تجاه الكوليستين من 2.6% إلى 4.7% في الفترة ذاتها. أمّا عن بكتريا الأسنيتوباكتر من مدينة سلطان الطبية العسكرية فقد ارتفعت نسبة المقاومة بها تجاه مضاد تيجاسيكلين من 1.3% إلى 6.6% بين العامين المعنيَّين، لم تُسجَل بها أي حالات مقاومة لمضاد الكوليستين. من جهة أخرى، سُجلت معظم المقاومة للتيجاسيكلين بمستشفى الملك فيصل التخصصي من عينات سريرية من وحدة العناية المركزة، بينما في مدينة الأمير سلطان العسكرية الطبية سُجلت المقاومة للتيجاسيكلين حصرياً من الأقسام خارج العناية المركزة. هذا ولم يُلحَظ أي تكتّل زمني لبكتريا الأسينيتوباكتر في أيّ من المستشفيين خلال فترة الدراسة.

خاتمة: سُجلت مقاومة الكوليستين و التيجاسيكلين في نسب مُعتبرة من الأسنيتوباكتر المتحصل عليها من عينات سريرية من اثنين من مستشفيات الرياض الكبرى خلال العامين مع ازدياد ملحوظ من عام إلى الآخر.

Objectives: To describe the rates and patterns of colistin and tigecycline resistance among *Acinetobacter baumannii* (*A. baumannii*) isolates from clinical specimens from 2 major hospitals in Riyadh Region over a 2-year period.

Methods: This is a retrospective review of records of all clinical isolates of *A. baumannii* from the departments of microbiology at King Faisal Specialist Hospital and Research Center (KFSHRC) and Prince Sultan Military Medical City (PSMMC), Riyadh, Kingdom of Saudi Arabia for the period from January 2010 to December 2011.

Results: Records for 1307 Acinetobacter species isolates were identified. The overall tigecycline resistance rates were 9.7% and colistin 1.8%. Among Acinetobacter isolates from KFSHRC, tigecycline resistance rate increased from 10.4% in 2010 to 20.5% in 2011. Colistin resistance increased over the same period from 2.6% to 4.7%. No Acinetobacter isolates from PSMMC were reported to be colistin resistant, while tigecycline resistance rates increased from 1.3% in 2010 to 6.6% in 2011. In KFSHRC, resistance to tigecycline was reported significantly more in isolates from samples that originated in the intensive care units, whereas in PSMMC tigecycline resistance was reported exclusively from clinical areas other than intensive care. No temporal clustering of Acinetobacter isolates was apparent in either hospital over the study period.

Conclusion: Tigecycline and colistin resistance were reported from a considerable proportion of Acinetobacter clinical isolates from the study hospitals over a 2-year period.

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Acinetobacter species are gram-negative coccobacilli, which were first described in 1911 as *Micrococcus calcoaceticus*. Their nomenclature had changed several times over the years before they became known as *Acinetobacter species* (*Acinetobacter spp.*) in the 1950s.[1] The natural habitat for *Acinetobacter spp.* is water and soil, but they have also been isolated from foods, arthropods, and the environment.[2] In humans, *Acinetobacter spp.* can colonize different body parts including the skin and respiratory tract. Some strains of *Acinetobacter spp.* can survive on surfaces for weeks, a characteristic that has promoted their transmission through environmental contamination in healthcare facilities.[1] *Acinetobacter species* infections have long been associated with wars and natural disasters. More recently however, they have been mostly implicated in outbreaks of infections in hospitals, especially in intensive care units.[3,4] The organism appears to possess an alarming ability to accumulate diverse mechanisms of resistance leading to the emergence of strains that are resistant to many of the commercially available antibacterial agents.[4] The lack of new antimicrobial agents in development complicates the matter even further.[5] Of great concern to clinicians and medical microbiologists are the often difficult to treat and intractable infections caused, in particular, by multi-drug resistant strains of *Acinetobacter.* Infections caused by *A. baumannii* have been associated with mortality rates as high as 52% in patients with bacteremia and 23-73% in those with pneumonia.[6] Most strains are resistant to penicillins, aminoglycosides, expanded spectrum cephalosporins and more recently fluoroquinolones.[6] The increased use of carbapenem for the treatment of infections caused by multi-resistant *A. baumannii* has resulted in increasing resistance to this class of antimicrobial agents.[7] It would therefore be not surprising to learn that *A. baumannii* were listed by the Infectious Diseases Society of America (IDSA) among the 6 top priority dangerous microorganisms.[5]

Infections caused by *A. baumannii* are not among the licensed indications for tigecycline.[8] However, several in-vitro reports, case series, and cohort studies have suggested that tigecycline and colistin may be effective in infections caused by carbapenem-resistant strains of *A. baumannii.*[9-14] Reduced *A. baumannii* susceptibility to these drugs has recently been reported from several countries across the world.[15-17] In one study, 78% of 82 Multidrug-resistant *A. baumannii* clinical isolates were either resistant or only intermediately susceptible to tigecycline.[18] Unfortunately, *A. baumannii* resistance to colistin has also emerged, thus rendering some isolates potentially without any active antimicrobial therapy options.[17,19] This study aims to determine the prevalence of tigecycline and colistin resistance over a 2-year period among *A. baumannii* clinical isolates from patients admitted to 2 major tertiary care hospitals in Riyadh, Saudi Arabia.

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Methods.

Isolate collection. A retrospective review of microbiology records at the departments of medical microbiology at King Faisal Specialist Hospital and Research Center (KFSHRC) and Prince Sultan Military Medical City (PSMMC), between January 2010 and December 2011, inclusive, was conducted to identify all clinical isolates of *A. baumannii* over that time period. The clinical isolates were classified according to their sample sources and patient locations. King Faisal Specialist Hospital and Research Center is a major tertiary care center in Riyadh City. Prince Sultan Military Medical City, also in Riyadh city, functions as an acute care hospital as well as a tertiary referral center for all Armed Forces Hospitals in the Kingdom of Saudi Arabia.

Inclusion and exclusion criteria. All *Acinetobacter spp*. clinical isolates at KFSHRC and PSMMC during the study period from all clinical specimen types (respiratory secretions, wound swabs, blood, urine, body fluids and deep tissues) were included. All *A. baumannii* strains isolated from surveillance and environmental samples were excluded. Duplicate isolates from the same patient and the same specimen type were also excluded.

Isolate identification. Samples were processed according to the standard laboratory procedures at the corresponding site. In the microbiology laboratory of KFSHRC, *A. baumannii* were identified on the basis of a combination of staining characteristics, colonial morphology, cytochrome oxidase reaction and automated identification system, VITEK 2 System (bioMerieux, Marcy l'Etoile, France). An overnight growth on blood agar of pure colonies is suspended in sterile saline to 0.5 McFarland Standard turbidity. The suspension is applied onto an ID GN Card N114 (bioMerieux, Marcy l'Etoile, France) and a result is obtained on VITEK 2 (bioMerieux, Marcy l'Etoile, France) within 8-12 hours. In the microbiology laboratory of PSMMC, isolates were identified on the basis of the their appearance on Gram stain, colonial morphology, cytochrome oxidase reaction, and by the API-NE kit (bioMerieux, Marcy l'Etoile, France). Pure

colonies obtained after overnight incubation on blood agar are suspended in sterile saline to 0.5 McFarland Standard and inoculated onto an API 20-NE kit (bioMerieux, Marcy l'Etoile, France). Results are available in 18-24 hours.

Antimicrobial susceptibility testing. In the microbiology laboratory of KFSHRC, colistin and tigecycline susceptibility testing is performed on VITEK 2 (bioMerieux, Marcy l'Etoile, France) inocula prepared by suspending colonies from overnight blood agar plates in sterile saline to the turbidity of a 0.5 McFarland standard. E-test strips (bioMerieux, Marcy l'Etoile, France) were used to verify colistin resistance in clinically significant multidrug-resistant Acinetobacter isolates for which no other therapeutic options were available. Colonies of the isolate being tested were suspended in sterile saline, adjusted to the density of a 0.5 McFarland standard, and streaked in 3 directions onto Mueller-Hinton agar plates (Saudi Prepared Media Laboratory, Riyadh, Saudi Arabia). Once the agar surface was completely dry, a colistin E-test strip (ranging from 0.06 to 1,024 mg/L) was applied onto it and the plate was incubated at 35°C for 18-24 hours. The minimum inhibitory concentration (MIC) is where the inhibition of growth intersected the E-test strip. As per Clinical and Laboratory Standards Institute (CLSI) recommendations, isolates with an MIC of ≤2 mg/L are considered susceptible (S) and those with MIC of >4 mg/L are considered resistant (R).[20] Tigecycline breakpoints were those recommended by the pharmaceutical product labelling in which isolates with an MIC of ≤2 mg/L are considered susceptible. In PSMMC Microbiology Department, colistin susceptibility testing was performed on Microscan automated system (Siemens, Sacramento, California). Resistant isolates were confirmed by E-test strips (bioMérieux, Marcy l'Etoile, France) following the same procedure and CLSI breakpoints as described above. Tigecycline breakpoints are also the same as cited earlier.

Ethical and the confidentiality consideration. An informed consent waiver form was completed and signed by the principle investigator. The confidentiality of the data was maintained by giving each clinical isolate a serial accession code number to preserve patient's privacy. The study was approved by the corresponding research ethics committees at KFSHRC & PSMMC.

Statistical analysis. Chi-square-test using the Statistical Package for Social Sciencs (IBM-SPSS Version 13, Chicago, USA) was used; differences resulting in P values of <0.05 were considered statistically significant.

Results. There was a total of 1,307 clinical isolates of *A. baumannii* from the 2 study hospitals over the 2-year period, including 610 isolates from KFSHRC and 697 isolates from PSMMC. Overall, the most common clinical source for the isolates was the respiratory tract (33%) followed by wounds (22.5%), blood (18.4%), and urine (12.5%). Cultures of other clinical samples, such as sterile body fluids, vascular catheter tips, and tissue samples contributed 13.7% of the isolates (**Table 1**).

Colistin and tigecycline resistance rates among all *A. baumannii* clinical isolates from the 2 hospitals were 1.8% and 9.7%, respectively (**Table 2**). There were however considerable differences in resistance rates and patterns between the 2 hospitals. Tigecycline resistance rate in *A. baumannii* from KFSHRC was almost 4 times higher than that in PSMMC (16.1% versus 4.2%). Remarkably, over the 2-year study period, rates of *A. baumannii* tigecycline resistance almost doubled (from 10.4 to 20.5%) in KFSHRC and increased by almost 5 folds (from 1.3 to 6.6%) in PSMMC. Moreover, colistin resistance was reported in 3.8% of all KFSHRC isolates; showing again a substantial increase over the 2-year period (from 2.6 to 4.7%). No colistin resistance was reported in any of the PSMMC *A. baumannii* isolates included in this study (**Figure 1**). Another interesting observation is the location of the patients from whom the resistant *A. baumannii* strains were isolated. In KFSHRC, colistin resistance rates were

Table 1 - *Acinetobacter baumannii* isolates from the 2 study sites by the sample type (N=1307 isolates).

Type of specimen	KFSHRC (610 isolates) n (%)	PSMMC (697 isolates) n (%)	Total (1307 isolates) n (%)
Respiratory tract	270 (44.3)	161 (23.1)	**431 (33.0)**
Wounds	124 (20.3)	170 (24.4)	**294 (22.5)**
Blood	99 (16.2)	141 (20.2)	**240 (18.4)**
Urine	80 (13.1)	83 (11.9)	**163 (12.5)**
Other	37 (6.1)	142 (20.4)	**179 (13.7)**
Total	**610 (46.7)**	**697 (53.3)**	**1307 (100)**

KFSHRC - King Faisal Specialist Hospital & Research Center, PSMMC - Prince Sultan Military Medical City

Table 2 - Colistin and tigecycline resistance rates among the study isolates by the study site (N=1307 isolate).

Variables	KFSHRC (610 isolates) n (%)	PSMMC (697 isolates) n (%)	Total (1307 isolates) n (%)
Colistin resistance	23 (3.8)	0 (0)	23 (1.8)
Tigecycline resistance	98 (16.1)	29 (4.2)	127 (9.7)

KFSHRC - King Faisal Specialist Hospital & Research Center, PSMMC - Prince Sultan Military Medical City

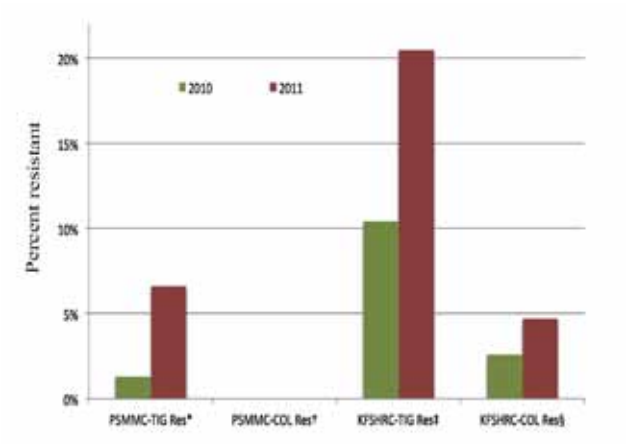

Figure 1 - Colistin and tigecycline resistance rates by hospital and year isolated. *Tigecycline resistant isolates from Prince Sultan Military Medical City (PSMMC), ‡Tigecycline resistant isolates from King Faisal Specialist Hospital & Research Center (KFSHRC), §Colistin resistant isolates from KFSHRC (colistin resistance was not reported in any isolates from PSMMC)

not significantly different amongst isolates from ICU and non-ICU patients (4.9% versus 3.1%, p=0.282), whereas tigecycline resistance in ICU was more than 3 times as much as that seen in isolates from non-ICU areas (27.9% versus 9.1%, p<0.001). In contrast, neither colistin nor tigecycline resistance were reported in any of the PSMMC ICU isolates, while tigecycline resistance was seen exclusively in non-ICU isolates (**Table 3**). There was no apparent temporal clustering of colistin or tigecycline resistance in either site (**Figure 2**).

Discussion. This is the first report of colistin and tigecycline resistance amongst clinical *A. baumannii* isolates from the Kingdom of Saudi Arabia. We report overall *A. baumannii* colistin and tigecycline resistance rates from the 2 major hospitals in Riyadh Region over a 2-year period of 1.8% and 9.7%. Resistance appeared to increase in both hospitals from one year to the next with significant differences in rates and patterns. Higher

Table 3 - *Acinetobacter baumannii* colistin and tigecycline resistance rates by patient location.

Isolates	Hospital/location					
	KFSHRC* (610 isolates) n (%)			PSMMC† (697 isolates) n (%)		
	ICU (226 isolates)	Non-ICU (384 isolates)	*P*-value	ICU (171 isolates)	Non-ICU (526 isolates)	*P*-value
Colistin resistant isolates	11 (4.9)	12 (3.1)	0.282	0 (0)	0 (0)	Not applicable
Tigecycline resistant isolates	63 (27.9)	35 (9.1)	<0.001	0 (0)	29 (5.5)	<0.001

KFSHRC - King Faisal Specialist Hospital & Research Center, PSMMC - Prince Sultan Military Medical City

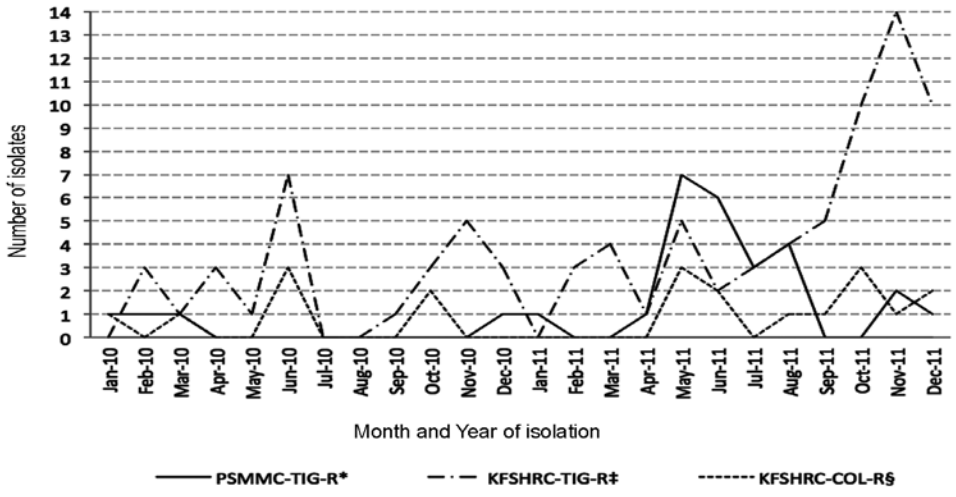

Figure 2 - Number of tigecycline or colistin resistant (COL-R) *Acinetobacter baumannii* isolates by the study site and month. *Tigecycline resistant (TIG-R) isolates from Prince Sultan Military Medical City (PSMMC), ‡Tigecycline resistant isolates from King Faisal Specialist Hospital & Research Center (KFSHRC), §Colistin resistant isolates from KFSHRC (colistin resistance was not reported in any isolates from PSMMC)

resistance rates have been reported from different parts of the world. For examples, tigecycline resistance rates ranging from 9.5% to 66% were reported in *A. baumannii* from the Arabian Gulf region, the Far East and South America.[18,21-23] Similarly, *A. baumannii* colistin resistance rates of 1% have been reported from Taiwan,[21] 12% from Kuwait,[22] and 27.9% from Korea.[24] The long-term use of colistin and tigecycline may result in selection of resistant strains effected through hetero-resistance in the former and hyper expression of the efflux pump in the latter.[17,19,25] A significantly higher proportion of *A. baumannii* isolates from patients in intensive care units (ICU) at KFSHRC were resistant to tigecycline than those isolated from non-ICU patients. Colistin resistance was slightly higher in ICU isolates than in non-ICU isolates, but the difference was not statistically significant. This can probably be partly explained by the more extensive use of broad-spectrum antimicrobials in ICU and the concentration of patients with more complex medical problems in such clinical areas. The opposite was however noted in PSMMC, where tigecycline is used infrequently in ICU and hence all Acinetobacter isolates resistant to this agent were isolated from non-ICU patients. Colistin resistance was not reported in any Acinetobacter isolate from PSMMC patients over the study period. The exact explanation for this is uncertain. It is of note however that KFSHRC functions almost exclusively as a tertiary referral center, regularly receiving patients who had spent long times as inpatient in other healthcare facilities across the Kingdom. Prince Sultan Military Medical City on the other hand provides acute as well as tertiary healthcare services to hospitals within the Medical Services Department of the Ministry of Defence. The patient population therefore reflects both acute unselected admissions in addition to patients with complex medical and surgical problems requiring highly specialized care.

This study has a number of limitations including those related to the considerable technical difficulties associated with tigecycline and colistin susceptibility testing. The disc diffusion method has been found to be inaccurate and not reproducible for colistin and tigecycline susceptibility testing of *A. baumannii*.[26] Agar dilution and broth microdilution are considered the gold standard susceptibility test methods for this organism. Both are cumbersome to perform and impractical to implement as routine tests in clinical laboratories.[20,26] E-test was used by both laboratories to verify colistin and tigecycline resistance. It has been known however that determining the exact MIC for colistin at the lower ranges (<0.5 mg/L) can be difficult due to the narrow angle of intersecting lines attributed to poor agar diffusion of colistin.[27] Kulah et al[28] found that results of *A. baumannii* colistin susceptibility testing using E-test methodology agreed with only 75.8% of those obtained using broth microdilution. Another contentious issue is the reported effect of the manganese concentration in the test media on MICs determined by E-test methodology. Tigecycline MICs determined on Mueller-Hinton agar containing manganese at concentrations higher than 8 mg/L may produce falsely elevated MICs, a problem which may occur if testing is performed on standard media.[29] Furthermore, when compared with broth microdilution, determining tigecycline MICs by E-tests can result in overestimation of nonsusceptibility.[30] Neither laboratory subjected their apparently resistant Acinetobacter isolates to reference laboratory testing; hence the rates reported cannot be considered certain. It is noteworthy that neither the CLSI nor the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have any published MIC breakpoints for *Acinetobacter spp.* susceptibility testing against tigecycline.[20,31] Clustering or outbreaks of infections caused by a limited number of resistant strains may result in over estimation of overall resistance rates. No obvious temporal clustering among the resistant isolates was noted in this study. Such a possibility cannot however be categorically excluded unless the strains are subjected to discriminatory epidemiological typing techniques.

In conclusion, we report tigecycline and colistin resistance rates among *Acinetobacter spp.* clinical isolates from 2 major Riyadh hospitals. The report highlights some of the technical challenges surrounding antimicrobial susceptibility testing, especially those that might have contributed to the results described here.

References

1. Schreckenberger P, Daneshvar M, Hollis D. Acinetobacter, Achromobacter, Chryseobacterium, Moraxella and other non-fermentative gram-negative rods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. Manual of clinical microbiology. 9th ed. Washington (DC): ASM Press; 2007. p. 770-802.
2. Fournier PE, Richet H, Weinstein RA. The Epidemiology and Control of Acinetobacter baumannii in Health Care Facilities. ***Clin Infect Dis*** 2006; 42: 692-699.
3. Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y, Carmeli Y. Multidrug-resistant *Acinetobacter baumannii*. ***Emerg Infect Dis*** 2005; 11: 22-29.
4. Lolans K, Rice TW, Munoz-Price LS, Quinn JP. Multicity outbreak of carbapenem-resistant *Acinetobacter baumannii* isolates producing the carbapenemase OXA-40. ***Antimicrob Agents Chemother*** 2006; 50: 2941-2945.

5. Talbot GH, Bradley J, Edwards JE, Gilbert D, Scheld M, Bartlett JG. Bad Bugs Need Drugs: An Update on the Development Pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. ***Clin Infect Dis*** 2006; 42: 657-668.
6. Jain R, Danziger LH. Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. ***Ann Pharmacother*** 2004: 38: 1449-1459.
7. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. ***Clin Microbiol Infect*** 2006; 12: 826-836.
8. TYGACIL® (tigecycline) For injection for intravenous use. [Updated: 2011; Accessed 2012 December 2). Available from URL: http://labeling.pfizer.com/showlabeling.aspx?id=491
9. Falagas ME, Rafailidis PI, Ioannidou E, Alexiou VG, Matthaiou DK, Karageorgopoulos DE, et al. Colistin therapy for microbiologically documented multidrug-resistant Gram-negative bacterial infections: a retrospective cohort study of 258 patients. ***Int J Antimicrob Agents*** 2010; 35: 194-199.
10. Gordon NC, Wareham DW. A review of clinical and microbiological outcomes following treatment of infections involving multidrug-resistant *Acinetobacter baumannii* with tigecycline. ***J Antimicrob Chemother*** 2009; 63: 775-780.
11. Karageorgopoulos DE, Kelesidis T, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant (including carbapenem-resistant) Acinetobacter infections: a review of the scientific evidence. ***J Antimicrob Chemother*** 2008; 62: 45-55.
12. Metan G, Alp E, Yildiz O, Percin D, Aygen B, Sumerkan B. Clinical experience with tigecycline in the treatment of carbapenem-resistant Acinetobacter infections. ***J Chemother*** 2010; 22: 110-114.
13. Michalopoulos AS, Karatza DC. Multidrug-resistant Gram-negative infections: the use of colistin. ***Expert Rev Anti Infect Ther*** 2010; 8: 1009-1017.
14. Vila J, Pachon J. Therapeutic options for *Acinetobacter baumannii* infections. ***Expert Opin Pharmacother*** 2008: 9: 587-599.
15. Iredell J, Thomas L, Power D, Mendes E. Tigecycline resistance in Australian antibiotic-resistant Gram-negative bacteria. ***J Antimicrob Chemother*** 2007; 59: 816-818.
16. Reid GE, Grim SA, Aldeza CA, Janda WM, Clark NM. Rapid development of *Acinetobacter baumannii* resistance to tigecycline. ***Pharmacotherapy*** 2007; 27: 1198-1201.
17. Rodriguez CH, Bombicino K, Granados G, Nastro M, Vay C, Famiglietti A. Selection of colistin-resistant *Acinetobacter baumannii* isolates in postneurosurgical meningitis in an intensive care unit with high presence of heteroresistance to colistin. ***Diagn Microbiol Infect Dis*** 2009; 65: 188-191.
18. Navon-Venezia S, Leavitt A, Carmeli Y. High tigecycline resistance in multidrug-resistant *Acinetobacter baumannii*. ***J Antimicrob Chemother*** 2007; 59: 772-774.
19. Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to Colistin in Multidrug-Resistant *Acinetobacter baumannii*. ***Antimicrob Agents Chemother*** 2006; 50: 2946-2950.
20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne (PA): Clinical and Laboratory Standards Institute; 2012.
21. Al-Sweih NA, Al-Hubail MA, Rotimi VO. Emergence of tigecycline and colistin resistance in Acinetobacter species isolated from patients in Kuwait hospitals. ***J Chemother*** 2011 23: 4.
22. Lee YT, Huang LY, Chiang DH, Chen CP, Chen TL, Wang FD, et al. Differences in phenotypic and genotypic characteristics among imipenem-non-susceptible Acinetobacter isolates belonging to different genomic species in Taiwan. ***Int J Antimicrob Agents*** 2009; 34: 580-584.
23. Tan TY, Ng LS. Susceptibility of multi-resistant gram-negative bacilli in Singapore to tigecycline as tested by agar dilution. ***Ann Acad Med Singapore*** 2007; 36: 4.
24. Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. ***J Antimicrob Chemother*** 2007; 60: 1163-1167.
25. Peleg AY, Adams J, Paterson DL. Tigecycline Efflux as a Mechanism for Nonsusceptibility in *Acinetobacter baumannii*. ***Antimicrob Agents Chemother*** 2007; 51: 2065-2069.
26. Gales AC, Reis AO, Jones RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. ***J Clin Microbiol*** 2001; 39: 183-190.
27. Gilad J, Giladi M, Poch F, Aharoni Y, Schwartz D. "All-in-one-plate" E-test and disk diffusion susceptibility co-testing for multiresistant *Acinetobacter baumannii*. ***Eur J Clin Microbiol Infect Dis*** 2006; 25: 799-802.
28. Kulah C, Celebi G, Aktas E, Mengeloglu Z, Comert F, Ankarali H. Unexpected tigecycline resistance among *Acinetobacter baumannii* Isolates: high minor error rate by Etest. ***J Chemother*** 2009; 21: 390-395.
29. Veenemans J, Mouton JW, Kluytmans JAJW, Donnely R, Verhulst C, van Keulen P. Effect of manganese in test media on in vitro bacterial susceptibility to tigecycline of *Enterobacteriaceae* and *Acinetobacter baumannii* to Tigecycline. ***J Clin Microbiol*** 2012; 50: 3077-3079.
30. Casal M, Rodriguez F, Johnson B, Garduno E, Tubau F, de Lejarazu RO, et al. Influence of testing methodology on the tigecycline activity profile against presumably tigecycline-non-susceptible *Acinetobacter spp*. ***J Antimicrob Chemother*** 2009; 64: 69-72.
31. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters (Version 2.0). 2012 [Updated 2012 January 1; Cited 2012 July 2]. Available from URL: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_2.0_120221.pdf