NEW MICROBIOLOGICA, **31**, 535-542, 2008

In vitro activity of tigecycline as a therapeutic option against multidrug-resistant *Acinetobacter* spp.

Özgen Köseoğlu Eser[1], Alper Ergin[2], Ferda Tunçkanat[1], Gülşen Hasçelik[1]

[1]Faculty of Medicine, Department of Microbiology and Clinical Microbiology;
[2]School of Health Services, Hacettepe University, Ankara, Turkey

SUMMARY

The study was performed to detect the in vitro activity of tigecycline in multidrug-resistant *Acinetobacter* isolates from patients in Hacettepe University Adult Hospital, Turkey. The microorganisms were isolated from clinical specimens of patients with respiratory and bloodstream infections. Thirty (66.7%) of the 45 inpatients were in ICUs. In vitro activity of imipenem, meropenem, ceftazidime, ciprofloxacin and aztreonam in 124 *Acinetobacter* species isolated was evaluated by microdilution test. Overall, 51 (41%) *Acinetobacter* spp. were found to be resistant to ≥3 antibiotics belonging to different antimicrobial classes and defined as multidrug-resistant (MDR). Among the MDR *Acinetobacter* spp. 32 (62.7%) were *Acinetobacter baumannii* and 19 (37.3%) *Acinetobacter lwoffii*. In vitro activity of tigecycline against MDR isolates were studied by E-test. Each MDR isolate was also tested for metallo-beta-lactamase (MBL) production using CLSI guidelines. Forty-five (88.2%) of the isolates were found to produce MBL**.** The MIC_{90}s of all antimicrobial agents tested except tigecycline were ≥64 µg/ml whereas the MIC_{50} and MIC_{90} of tigecycline were found 1 µg/ml and 1.5 µg/ml, respectively. ERIC-PCR results revealed that bloodstream and respiratory isolates had nine and six different patterns, respectively. In conclusion, tigecycline has been shown to have potent in vitro activity against MDR *Acinetobacter* spp. and might be of therapeutic value in the treatment of infections due to MDR *Acinetobacter* spp., including those harbouring MBLs. Further clinical trials are needed to confirm the efficacy of tigecycline in the management of MDR *Acinetobacter* infections.

KEY WORDS: Acinetobacter, Multidrug-resistance, Glycylcycline, Metallo-beta-lactamases

Received March 03, 2008 *Accepted* June 09, 2008

INTRODUCTION

Acinetobacter is an important gram-negative, non-fermentative pathogen that can give rise to nosocomial infections especially in intensive care units (ICU's) (Jain and Danziger, 2004). *Acinetobacter* species mainly cause pneumonia and bacteremia in hospital setting with high mortality and morbidity rates (Rossolini *et al.,* 2007). *Acinetobacter* is resistant to many antimicrobial agents belonging to different classes and increased antimicrobial resistance of this organism makes its treatment difficult (McGowan, 2006). Although carbapenems are known to be one of the very few antimicrobial agents showing consistent activity against *Acinetobacter*, the emergence of carbapenem-resistant *Acinetobacter* strains leaves the drug out of utility. Carbapenem resistance in *Acinetobacter* occurs by beta-lactamase production like metallo-beta-lactamases (MBL) or oxacillinases, porin loss or modification of penicillin-binding proteins, but it is mainly attributed to production of carbapenemases (Poirel and Nordmann, 2006). Increasing reports of carbapenem-resistance in *Acinetobacter* requires exploration of new antibiotics.

Corresponding author
Özgen Köseoğlu Eser, MD/PhD
Associate Professor of Infectious Diseases
Hacettepe University, Faculty of Medicine
Department of Microbiology and Clinical Microbiology
Ankara, 06100 Turkey
E-mail: ozgen@tr.net

Tigecycline is the first commercially available glycylcycline which was licensed by the USA's Food and Drug Administration in June 2005.
Tigecycline presents a wide spectrum of antimicrobial activity for the treatment of multidrug-resistant infections including *Acinetobacter* spp. (Rose e*t al.,* 2006).
This study was designed to assess the in vitro activity of tigecycline against multidrug-resistant clinical strains of *Acinetobacter* spp. isolated from patients in a tertiary 850-bed university hospital in Ankara, Turkey. We also tested the presence of MBL in multidrug-resistance *Acinetobacter* spp. to see the activity of tigecycline despite the presence of MBLs.

MATERIALS AND METHODS

Bacterial strains

A total of 124 *Acinetobacter* spp. obtained from clinical specimens of patients admitted to Hacettepe University Hospital between January and December 2003 were included in the study. Only one isolate per patient was accepted in the study. Species identification was performed by Gram staining, oxidase test and Sceptor system (Becton Dickinson, USA).

Antimicrobial susceptibility testing

Antimicrobial agents tested against 124 *Acinetobacter* isolates were imipenem, meropenem, ciprofloxacin, ceftazidime, aztreonam and tigecycline. The MIC values of antibiotics other than tigecycline were determined by using the broth microdilution method of the Clinical and Laboratory Standarts Institute (CLSI, 2006). Susceptibility for the antimicrobial agents was determined according to the interpretive criteria of CLSI, 2006. MIC testing for tigecycline in MDR isolates was performed by E-test using a standardized 0.5 McFarland inoculum on freshly prepared Mueller-Hinton agar plates (BBL,USA). As there were no interpretive criteria approved for tigecycline while testing *Acinetobacter* spp., the MIC values were interpreted according to the instructions provided by the manufacturer (AB Biodisk, Solna, Sweden) at the point of 80% inhibition. Interpretive criteria for tigecycline MICs were determined according to the US Food and Drug Administration-approved criteria, susceptibility at ≤2 μg/μl and resistance ≥8 μg/μl, applied for *Enterobacteriaceae* (Wyeth Pharmaceuticals, 2005). *Escherichia coli* ATCC 25922 was used as a quality control for E-test.

Multidrug-resistance

Multidrug-resistance was defined as resistance to three or more classes of antimicrobial agents. These classes were carbapenems (imipenem, meropenem), cephalosporins (ceftazidime), fluoroquinolones (ciprofloxacin), monobactams (aztreonam). Tigecycline was not included in the determination of multi-resistant *Acinetobacter* spp. as interpretive criteria are not available.

Metallo-beta-lactamase production

Imipenem and EDTA disk diffusion combined test was used to detect MBL production. *Acinetobacter* isolates were inoculated onto Mueller-Hinton agar as described for the disk diffusion method by CLSI guidelines (CLSI, 2006). Two separate imipenem (10 μg) disks (Becton-Dickinson, USA) were placed on an agar plate and 10 μl of a 0.5 M EDTA solution (pH 8.0) (Sigma, Germany) was added to one of the imipenem disks. After an overnight incubation at 35°C, the inhibition zones around the imipenem disks with and without EDTA were measured and compared with each other. An increase of ≥7 mm in the zone diameter for imipenem in the presence of EDTA was interpreted as a positive test result.

Enterobacterial repetitive consensus PCR

Enterobacterial repetitive consensus (ERIC)-PCR was conducted in 25 μl volume, each containing 2 μl of genomic DNA from 51 MDR *Acinetobacter* clinical isolates, 25 mM $MgCl_2$, 30 pM of ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3'), 30 pM of ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'), 1 U of Taq polymerase (Qiagen, Germany), 0,2 mM of each dNTP. Amplification was carried out with the following program: 95ºC for 5 min followed by 5 cycles of 1 min at 94ºC, 1 min at 30ºC, 1 min at 72ºC and 40 cycles of 1 min at 94ºC, 1 min at 52ºC, 1 min at 72ºC. A final extension step was run at 72ºC for 5 min.
The amplified PCR products were electrophoresed in 2% agarose gels with 1xTAE running buffer at 110 V for 2 and a half hours. Φx174 HaeIII molecular marker was used as a size marker to calculate the absolute lengths of the

bands in individual sample lanes in all gels. After the gels were stained with ethidium bromide, the bands were examined by Ultra Violet Products (UVP) White /UV transilluminator and Grab-IT 2.0 annotating grabber programme.
The isolates were categorized as " Identical" if their patterns had the same numbers of bands and as "Different" based on a single band difference.

RESULTS

Patient characteristics

A total of 51 (41%) A*cinetobacter* isolates out of 124 were found to be multidrug-resistant. Six (11.8%) of these strains were isolated from outpatients and 45 (88.2%) of them were isolated from inpatients. Thirty (66.7%) of the inpatients were from ICUs of internal medicine, general surgery, thoracic surgery, cardiovascular surgery and anesthesiology. None of the MDR strains were isolated from the same patient.
Among the multidrug-resistant *Acinetobacter* spp. 32 (62.7%) isolates were identified as *A. baumannii* and 19 (37.3%) as *A. lwoffii*. They were isolated from bronchoalveolar lavage (BAL) (n=23), blood (n=16), central-venous catheters (CVC) (n=7), sputum (n=4) and bone-marrow (n=1) specimens.

Antibiotic susceptibility

All of the multidrug-resistant isolates were resistant to carbapenem; imipenem, fluoroquinolones; ciprofloxacin and cephalosporin; ceftazidime. The in vitro activities, MIC_{50} and MIC_{90} values of the antimicrobial agents tested against *Acinetobacter* spp. are presented in Table 1. MIC_{90}s of all antimicrobial agents tested, were ≥64 µg/ml except tigecycline (Table 1). Tigecycline was the only antimicrobial agent which displays a good activity against MDR strains with MIC_{90} of 1.5 µg/ml. Forty-five (88.2%) of the isolates were found to have MBL production. Tigecycline did not appear to be affected by the presence of MBL.

ERIC-PCR analysis

Fifty one clinical isolates, when evaluated with ERIC-PCR, generated distinct bands varying from 7 to 11 bands. Due to variation in characteristics of blood/CVC/bone marrow and bronchoalveolar lavage/sputum samples, they were evaluated in separate Tables (Tables 2 and 3). Nine ERIC-PCR patterns A-E (*A. baumannii*) and T-W (*A. lwoffii*) represented MDR *Acinetobacter* spp. among blood/CVC/bone marrow isolates according to hospital location (Table 2). MDR *Acinetobacter* spp. from bronchoalveolar lavage/sputum samples showed 6 ERIC-PCR patterns F-H (*A. baumannii*) and X-Z (*A. lwoffii*) according to hospital location (Table 3).

DISCUSSION

Acinetobacter spp. can cause infections in the hospital setting especially in ICUs. The most frequent manifestations of *Acinetobacter* infections are respiratory and bloodstream infections associated with outbreaks. *Acinetobacter* infections can cause an increased morbidity and mortality fa-

TABLE 1 - *Antimicrobial susceptibilities of multidrug-resistant Acinetobacter spp. (n=51).*

Antimicrobial agent	*MIC_{50} (µg/ml)*	*MIC_{90} (µg/ml)*	*Range (µg/ml)*	*Resistance %*
Imipenem	64	128	8-256	100
Meropenem	32	64	4-256	96.1
Ciprofloxacin	64	128	64-256	100
Ceftazidime	128	256	32-256	100
Aztreonam	128	256	8-256	98
Tigecycline	1	1.5	0.094-2	-

cilitating poor prognosis (Rossolini *et al.,* 2007). In the present study, 27 (52.9%) of the patients had respiratory and 24 (47.1%) had bloodstream infections. A total of 45 (88.2%) *Acinetobacter* infections were identified >48 hours after the patients were admitted to the hospital and thus met the criteria for nosocomial infection (Masterton *et al.,* 2007). Although community acquired

TABLE 2 - *Characteristics of Acinetobacter spp. isolated from blood/CVC/bone marrow samples.*

Isolate	*Date of isolation*	*Sex*	*Site of isolation*	*Ward*	*Species*	*ERIC-PCR*
1	16/01/2003	M	Blood	GSICU	A. lwoffii	T
2	30/01/2003	M	Blood	CVSICU	A. lwoffii	U
3	07/02/2003	F	Blood	IMICU	A. baumannii	C
4	28/02/2003	F	Bone marrow	IMICU	A. lwoffii	T
5	28/02/2003	F	CVC	IMICU	A. baumannii	A
6	12/03/2003	M	Blood	GS	A. lwoffii	U
7	24/03/2003	F	Blood	IM	A. baumannii	B
8	15/04/2003	M	Blood	GSICU	A. lwoffii	U
9	17/04/2003	M	Blood	Outpatient	A. lwoffii	W
10	17/04/2003	F	Blood	IMICU	A. lwoffii	U
11	24/04/2003	F	Blood	Outpatient	A. baumannii	D
12	28/04/2003	F	CVC	Outpatient	A. baumannii	B
13	13/05/2003	F	CVC	AICU	A. baumannii	D
14	30/05/2003	F	Blood	IMICU	A. lwoffii	W
15	13/06/2003	M	CVC	IMICU	A. baumannii	D
16	25/06/2003	M	Blood	IMICU	A. lwoffii	W
17	14/07/2003	M	CVC	GS	A. baumannii	D
18	28/07/2003	F	Blood	Outpatient	A. lwoffii	V
19	07/08/2003	F	CVC	IM	A. baumannii	D
20	11/08/2003	F	Blood	IM	A. baumannii	E
21	29/08/2003	F	Blood	IM	A. lwoffii	W
22	03/09/2003	M	Blood	IM	A. baumannii	D
23	05/09/2003	M	Blood	IMICU	A. baumannii	D
24	03/11/2003	F	CVC	Outpatient	A. baumannii	D

CVC: cardiovascular catheters; Al: A. lwoffii; ICU: Intensive care unit; IM: internal medicine; GS: general surgery; CVS: cardiovascular surgery; A: anesthesiology.

TABLE 3 - *Characteristics of Acinetobacter spp. isolated from bronchoalveolar lavage/sputum samples.*

Isolate	*Date of isolation*	*Sex*	*Site of isolation*	*Ward*	*Species*	*ERIC-PCR*
1	28/01/2003	F	Sputum	IM	A. baumannii	F
2	20/02/2003	M	BAL	Neurology	A. baumannii	F
3	26/02/2003	F	BAL	IMICU	A. baumannii	F
4	03/03/2003	M	BAL	GSICU	A. baumannii	F
5	19/03/2003	F	BAL	GS	A. lwoffii	X
6	21/03/2003	F	BAL	IMICU	A. lwoffii	X
7	27/03/2003	M	BAL	IMICU	A. lwoffii	X
8	28/03/2003	M	BAL	IMICU	A. baumannii	F
9	18/04/2003	M	BAL	IMICU	A. lwoffii	X
10	18/04/2003	F	Sputum	IM	A. baumannii	F
11	24/04/2003	F	BAL	IMICU	A. lwoffii	Y
12	28/04/2003	M	BAL	IM	A. baumannii	F
13	01/05/2003	M	BAL	IMICU	A. baumannii	F
14	05/05/2003	M	BAL	IMICU	A. baumannii	F
15	22/05/2003	F	BAL	IMICU	A. baumannii	F
16	29/05/2003	M	Sputum	AICU	A. baumannii	G
17	02/06/2003	M	BAL	IMICU	A. baumannii	F
18	23/06/2003	M	Sputum	IMICU	A. baumannii	F
19	24/06/2003	F	BAL	GSICU	A. lwoffii	X
20	25/06/2003	F	BAL	IMICU	A. baumannii	F
21	11/07/2003	M	BAL	IMICU	A. baumannii	H
22	14/07/2003	F	BAL	IMICU	A. baumannii	F
23	29/07/2003	F	BAL	Outpatient	A. lwoffii	Z
24	05/08/2003	F	BAL	IM	A. baumannii	F
25	05/08/2003	M	BAL	GSICU	A. baumannii	F
26	11/08/2003	F	BAL	IM	A. baumannii	F
27	14/08/2003	F	BAL	IM	A. lwoffii	X

BAL: bronchoalveolar lavage; Al: A. lwoffii; ICU: Intensive care unit; IM: internal medicine; GS: general surgery; A: anesthesiology.

Acinetobacter infections are uncommon, they are increasingly recognized (Dijkshoorn *et al.,* 2007; Falagas *et al.,* 2007). In the present study, only six (11.8%) community-acquired MDR *Acinetobacter* infections were detected in the study population. Multidrug-resistance in *Acinetobacter* spp. is on rise all over the world (Gaynes *et al.,* 2005; Coelho *et al.,* 2006; Falagas *et al*., 2006). Carbapenem-resistant *Acinetobacter baumannii* infections are associated with prolonged ICU or hospital stay (Lotholary *et al.,* 1995; Smolyakov *et al.,* 2003; Playford *et al.,* 2007). In our study, 30 (58.8%) of the patients were hospitalized in ICUs and 15 (29.4%) of the patients in other wards which indicates this correlation.

Carbapenems being a last line of defense in *Acinetobacter* infections become a powerful selector in multidrug-resistance. In *Acinetobacter* spp., carbapenem resistance mainly occurs by production of OXA carbapenemases or more rarely, MBLs (Livermore D., 2005 and Poirel L. and Nordmann P., 2006). In our study, of 51 MDR *Acinetobacter* isolates, 45 (88.2%) were found as MBL producers, but all the isolates were resistant to imipenem. MBL production is typically associated with resistance to aminoglycosides and quinolones (Cornaglia *et al.,* 2007). In the present study, all MBL-producing isolates were also resistant to ciprofloxacin. Therapeutic options available to treat these multidrug-resistant *Acinetobacter* infections are limited. Acquisition of beta-lactamase genes which plays a significant role in multidrug-resistance of *Acinetobacter* isolates leaves polymixins as the mere drug in treatment. However, polymixins are not clinically available agents because of their toxicity (Falagas M.E. and Kasiakou S.F., 2005). Although in vitro synergy studies indicate the efficiency of combination therapies for these infections (Timurkaynak *et al.,* 2006), the role of monotherapy versus combination therapy is unclear and needs to be confirmed by clinical trials that aim to identify the most appropriate regimens for the treatment of *Acinetobacter* infections (Murray and Hospenthal, 2005; Peleg, 2007).

Tigecycline is a novel antibiotic being the first member of a new class of glycylcyclines. It was approved by the US Food and Drug Administration for the treatment of complicated skin and soft tissue infections and complicated intra-abdominal infections (Rice L.B., 2006). Tigecycline has a potent activity against a variety of gram-positive and gram-negative bacteria including multidrug-resistant gram-negative bacilli (Rose *et al.,* 2006). Tigecycline also appears to be a promising agent for the treatment of multidrug-resistant *Acinetobacter* infections in various studies (Seifert *et al.,* 2006; Ratnam *et al.,* 2006; Tiengrim *et al.,* 2006; Hoban *et al.,* 2007; Halstead *et al.,* 2007). In the present study, tigecycline demonstrated a good in vitro activity against multidrug-resistant *Acinetobacter* isolates with MIC_{50} and MIC_{90} values of 1 µg/ml and 1.5 µg/ml, respectively. The MIC_{90} values of tigecycline among the studied isolates for respiratory and bloodstream infections were different. Although the MIC_{50}s were the same (1 µg/ml) for both groups, MIC_{90}s were found 1.5 µg/ml and 2 µg/ml, respectively. When we evaluated the relationship between the highest MIC value (2 µg/ml) with the site of infection/ward of inpatients/bacterial species and sensitivity to tigecycline in our MDR isolates, we found that three were from bloodstream and two were from respiratory samples. Two of the three bloodstream samples and one of the two respiratory samples were from patients in ICUs. Among these isolates, one bloodstream and one respiratory isolate were *A. baumannii*. This data does not indicate a relationship between parameters considered since we do not have any resistant isolates against tigecycline. So tigecycline may be considered an alternative therapeutic option that can be used empirically for patients with respiratory infections when other treatment options are not available.

In this study, the genotypic relatedness of MDR *Acinetobacter* isolates was investigated by the molecular typing method, ERIC-PCR. It was used to show the genotype patterns of our MDR *Acinetobacter* spp. since it can generate a characteristic genomic fingerprint which can be used to determine intra- and inter-species genotypic variations among *Enterobacteriaceae*, *P.aeruginosa* and *A. baumannii* (Jeong *et al.,* 2006). ERIC-PCR analysis showed common genotypic patterns among isolates of our patients (eg. -D pattern in bloodstream isolates) hospitalized in different wards at different times. These data could not be attributed to an outbreak as it has been proposed that strains with these characteristics circulate in the community and are selected in hospitals

through selective antibiotic pressure (Dijkshoorn *et al.* 1996).

MBL production of *Acinetobacter* isolates in a hospital setting requires surveillance data for the selection of empiric therapy and monitoring of resistance control. Our results which are adequate to alert us to restrict the overuse of carbapenem in ICUs indicate that this attempt should be taken as a priority to prevent drug pressure resulting in the development of new resistance in the hospital environment. Tigecycline presents itself as a promising agent to limit the spread of these pathogens. Tigecycline has a potent in vitro activity and might have useful therapeutic activity in patients with respiratory infections due to multidrug-resistant *Acinetobacter* spp, including those harbouring MBLs. However, further clinical studies are still required to evaluate its efficacy especially in the treatment of invasive infections.

ACKNOWLEDGEMENTS

This study was supported with a grant by Hacettepe University Scientific Researches Unit, Ankara, Turkey (Project no: 04D05101001). We thank Wyeth Pharmaceuticals, Turkey for providing the tigecycline E-test strips.

REFERENCES

CLSI, Clinical And Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 16th Informational Supplement. M100-S16. Wayne, PA USA. (2006).

Coelho J.M., Turton J.F., Kaufmann M.E., Glover J., Woodford N., Warner M., Palepou M., Pike R., Pitt T.L., Patel B.C., Livermore D.M. (2006). Occurence of carbapenem-resistant *Acinetobacter baumannii* clones at multiple hospitals in London and Southeast England. *J. Clin. Microbiol*. **44** (10), 3623-3627.

Cornaglia G., Akova M., Amicosante G., Canton R., Cauda R., Docquier J-D., Edelstein M., Frere J-M., Fuzi M., Galleni M., Giamarellou H., Gniadkowski M., Koncan R., Libisch B., Luzzaro F., Miriagou V., Navarro F., Nordmann P., Pagani L., Peixe L., Poirel L., Souli M., Tacconelli E., Vatopoulos A., Rossolini G.M., on Behalf Of Escmid Study Group for Antimicrobial Resistance Surveillance (ESGARS). (2007). Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens:open issues. *Int. J. Antimicrob. Agents*. **29** (4), 380-388.

Dijkshoorn L., Nemec A., Seifert H. (2007). An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumanii*. *Nat. Rev. Microbiol*. **5** (12), 939-951.

Dijkshoorn L., Aucken H., Gerner-Smidth P., Janssen P., Kaufmann M.E., Garaizar J., Ursing J., Pitt T.L. (1996). Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. *J. Clin. Microbiol.* **34**, 1519-1525.

Falagas M.E., Karveli E.A., Kelesidis I., Kelesidis T. (2007). Community-acquired *Acinetobacter* infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **26** (12), 857-868.

Falagas M.E., Kasiakou S.F. (2005). Colistin: the revival of polymixins for the management of multidrug-resistant Gram-negative bacterial infections. *Clin. Infect. Dis*. **40** (9), 1333-1341.

Falagas M.E., Koletsi P.K., Bliziotis I.A. (2006). The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *J. Med. Microbiol*. **55** (12), 1619-1629.

Gaynes R., Edwards J.R., National Nosocomial Infections Surveillance System (2005). Overview of nosocomial infections caused by gram-negative bacilli. *Clin. Infect. Dis.* **41** (6), 848-854.

Halstead D.C., Abid J., Dowzicky M.J. (2007). Antimicrobial susceptibility among *Acinetobacter calcoaceticus-baumannii* complex and Enterobacteriaceae collected as part of the tigecycline evaluation and surveillance trial. *J. Infect.* **55** (1), 49-57.

Hoban D.J., Bouchillon S.K., Dowzicky M.J. (2007). Antimicrobial susceptibility of extended-spectrum beta-lactamase producers and multidrug-resistant *Acinetobacter baumannii* throughout the United States and comparative in vitro activity of tigecycline, a new glycylcycline antimicrobial. *Diag. Microbiol. Infect. Dis*. **57** (4), 423-428.

Jain R., Danziger L.H. (2004). Multidrug-resistant *Acinetobacter* infections: An emerging challenge to clinicians. *Ann. Pharmacother.* **38** (9), 1449-1459.

Jeong S.H., Bae I.K., Park K.O., An Y.J., Sohn J.G., Jang S.J., Sung K.H., Yang K.S., Lee K., Young D. and Lee S.H. (2006). Outbreaks of Imipenem-Resistant *Acinetobacter baumannii* producing carbapenemases in Korea. *J. Microbiol.* **44** (4), 423-431.

Livermore D. (2005). Tigecycline: what is it and where should it be used? *J. Antimicrob. Chemother.* **56**, 611-614.

Lotholary O., Fagon J.Y., Hoi A.B., Slama M.A, Pierre J. Giral P., Rosenzweig R., Gutmann L., Safar M., Acar J. (1995). Nosocomial acquisition of multiresistant Acinetobacter baumannii: risk factors and prognosis. *Clin. Infect. Dis.* **20** (4), 790-796.

Masterton R., Craven D., Rello J, Struelens M.,

FRIMODT-MOLLER N., CHASTRE J, ORTQVIST A, COGNALIA G, LODE H., GIAMARELLOU H., BONTEN M.J., ERAKSOY H., DAVEY P. (2007). Hospital-acquired pneumonia guidelines in Europe: a review of their status and future development. *J. Antimicrob. Chemother*. **60** (2), 206-213.

MCGOWAN J.E. (2006). Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. *Am. J. Infect. Control*. **34** (5 Suppl 1), 29-37.

MURRAY C.K., HOSPENTHAL D.R. (2005). Treatment of multidrug-resistant *Acinetobacter*. *Curr. Opin. Infect. Dis*. **18** (6), 502-506.

PELEG A.Y. (2007). Optimizing therapy for *Acinetobacter baumannii*. *Semin. Respir. Crit. Care Med.* **28** (6), 662-671.

PLAYFORD E.G., CRAIG D.C., IREDELL J.R. (2007). Carbapenem-resistant *Acinetobacter baumannii* in intensive care unit patients: risk factors for acquisition, infection and their consequences. *J. Hosp. Infect.* **65** (3), 204-211.

POIREL L., NORDMANN P. (2006). Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin. Microbiol. Infect.* **12** (9), 826-836.

RICE L.B. (2006). Challenges in identifying new antimicrobial agents effective for treating infections with *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Clin. Infect. Dis*. **43** (Suppl 2), 100-105.

ROSE W.E., RYBAK M.J. (2006). Tigecycline: first of a new class of antimicrobial agents. *Pharmacother*. **26** (8), 1099-1110.

ROSSOLINI G.M., MANTENGOLI E., DOCQUIER J.D., MUSMANNO R.A., RATNAM I., FRANKLIN C., SPELMAN D.W. (2007). In vitro activities of new and conventional antibiotics against multi-drug resistant Gram negative bacteria from patients in the intensive care units. *Pathology.* **39** (6), 586-588.

ROSSOLINI G.M., MANTENGOLI E., DOCQUIER J-D, MUSMANNO R.A., CORATZA G. (2007). Epidemiology of infections caused by multiresistant Gram-negatives: ESBLs, MBLs, panresistant strains. *New Microbiologica.* **30** (3), 332-339.

SEIFERT H., STEFANIK D., WISPLINGHOFF H. (2006). Comparative in vitro activities of tigecycline and 11 other antimicrobial agents 215 epidemiologically defined multidrug-resistant *Acinetobacter baumannii* isolates. *J. Antimicrob. Chemother.* **58** (5), 1099-1100.

SMOLYAKOV R., BORER A., RIESENBERG K., SCHLAEFFER F., ALKAN M., PORATH A., RIMAR D., ALMOG Y., GILAD Y. (2003). Nosocomial multi-drug resistant Acinetobacter baumannii infection: risk factors and outcome with ampicillin-sulbactam treatment. *J. Hosp. Infect.* **54** (1), 32-38.

TIENGRIM S., TRIBUDDHARAT C., THAMLIKITKUL V. (2006). In vitro activity of tigecycline against clinical isolates of multidrug-resistant *Acinetobacter baumannii* in Siriraj Hospital, Thailand. *J. Med. Assoc. Thai*. **89** (Suppl 5), 102-105.

TIMURKAYNAK F., CAN F., KURT AZAP O., DEMIRBILEK M., ARSLAN H., KARAMAN S.O. (2006). In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from intensive care units. *Int. J. Antimicrob. Agents*. **27** (3), 224-228.