original article

Risk factors for infection with colistin-resistant gram-negative microorganisms: a multicenter study

Gul R. Yilmaz,[a] Murat Dizbay,[b] Tumer Guven,[c] Husnu Pullukcu,[d] Meltem Tasbakan,[d] Ozlem Tunccan Guzel,[b] Yasemin T. Tekce,[e] Mehmet Ozden,[f] Ozge Turhan,[g] Rahmet Guner,[c] Yasemin Cag,[h] Fatma Bozkurt,[i] Fatma Yilmaz Karadag,[j] Elif Doyuk Kartal,[k] Gokhan Gozel,[l] Cemal Bulut,[m] Sebnem Erdinc,[m] Siran Keske,[n] Ziya Cibali Acikgoz,[o] Mehmet A. Tasyaran[c]

From the [a]TR Ministry of Health Ankara Ataturk Training and Research Hospital, Infectious Diseases and Clinical Microbiology, Ankara, Turkey; [b]Department of Infectious Diseases and Clinical Microbiology, Gazi University Faculty of Medicine, Ankara, Turkey; [c]Department of Infectious Diseases and Clinical Microbiology, Yildirim Beyazit University, Medical Faculty, Ankara, Turkey; [d]Department of Infectious Diseases and Clinical Microbiology, Ege University, Faculty of Medicine, Izmir, Turkey; [e]Türkiye Yuksek Ihtisas Training and Research Hospital, Infectious Diseases and Clinical Microbiology, Ankara, Turkey; [f]Department of Infectious Diseases and Clinical Microbiology, Firat University, Medical Faculty, Elazig, Turkey; [g]Department of Infectious Diseases and Clinical Microbiology, Akdeniz University, Medical Faculty, Antalya, Turkey; [h]Department of Infectious Diseases and Clinical Microbiology, Lutfi Kirdar Training and Research Hospital, Istanbul, Turkey; [i]Department of Infectious Diseases and Clinical Microbiology, Diyarbakır Training and Research Hospital, Diyarbakir, Turkey; [j]Department of Infectious Diseases and Clinical Microbiology, Istanbul Medeniyet University, Faculty of Medicine, Goztepe Training and Research Hospital, Istanbul, Turkey; [k]Department of Infectious Diseases and Clinical Microbiology, Osman Gazi University, Faculty of Medicine, Eskicehir, Turkey; [l]Department of Infectious Diseases and Clinical Microbiology, Cumhuriyet University, Faculty of Medicine, Sivas, Turkey; [m]Department of Infectious Diseases and Clinical Microbiology, Ankara Training and Research Hospital, Ankara, Turkey; [n]Department of Infectious Diseases and Clinical Microbiology, Giresun University, Faculty of Medicine, Giresun, Turkey; [o]Department of Microbiology, Yildirim Beyazit University Medical Faculty, Ankara, Turkey

Correspondence: Associate Prof. Gul R. Yilmaz · TR Ministry of Health, Ankara Ataturk Training and Research Hospital, Infectious Diseases and Clinical Microbiology, Bilkent Cad. No. 3 Ankara 06800, Turkey · T: 00903122912525, F: 00903124269393 · rruhsar6@yahoo.com · ORCID ID: orcid.org/0000-0001-5878-4301

Ann Saudi Med 2016; 36(3): 216-222

DOI: 10.5144/0256-4947.2016.216

BACKGROUND: Knowing risk factors for colistin resistance is important since colistin is the only remaining choice for the treatment of infections caused by multi-drug resistant microorganisms.
OBJECTIVE: Evaluate risk factors associated with infection by colistin-resistant microorganisms.
DESIGN: Retrospective study.
SETTINGS: Tertiary healthcare centers.
PATIENTS AND METHODS: An e-mail including the title and purpose of the study was sent to 1500 infectious disease specialists via a scientific and social web portal named "Infeksiyon Dunyasi (Infection World)". Demographic and clinical data was requested from respondents.
MAIN OUTCOME MEASURE(S): Colistin-resistance.
RESULTS: Eighteen infectious disease specialists from twelve tertiary care centers responded to the invitation. Data was collected on 165 patients, 56 cases (39.9%) and 109 (66.0%) age- and sex-matched controls. The colistin-resistant microorganisms isolated from cases were 29 *Acinetobacter baumannii* (51.8%), 18 *Pseudomonas aeruginosa* (32.1%) and 9 *Klebsiella spp.* Colistin, carbapenem, and quinolone use in the last three months were risk factors for colistin resistance in the univariate analysis. Previous quinolone use in the last three months (*P*=.003; RR:3.2; 95% CI:1.5-6,7) and previous colistin use in the last three months (*P*=.001; RR: 3.6; 95% CI: 1.63-7.99) were significant risk factors in the multivariate analysis.
CONCLUSION: Clinicians should limit the use of quinolones and remain aware of the possibility of resistance developing during colistin use.
LIMITATIONS: The lack of a heteroresistance analysis on the isolates. No data on use of a loading dose or the use of colistin in combination.

The increased prevalence of infections related to multi-resistant microorganisms has led to a search for alternative antibiotics in recent years. Colistin, which was first used in clinical practice in the 1960s, was abandoned due to nephrotoxicity and neurotoxicity. However, in the last fifteen years, colistin has gained an important role as salvage therapy for patients infected with microorganisms susceptible only to colistin.[1] As with all antibiotics, the increased use of colistin has resulted in the development of colistin resistance.[2-7]

Adaptive or mutational mechanisms may play a role in resistance to colistin.[8,9] In colistin resistance, different gene mutations cause resistance by changing the outer membrane of gram-negative bacteria. Although data on resistance mechanisms is limited, the regulator systems of PmrA-PmrB and PhaP-PhOq are known to play a key role in resistance development.[10,11]

Colistin-resistant *Acinetobacter spp* was first reported in the Czech Republic as 5.9% in 1999; in subsequent years resistance increased to as high as 40% in some reports.[12,13] In studies in Asia, Europe and North and South America, the rate of colistin resistance in *A baumannii* has generally been below 7%.[14-17] However, in studies conducted in Bulgaria and Spain, rates of colistin resistance of 16.7% and 19.1%, respectively, were reported for *A baumannii*.[18,19] Colistin resistance in *A baumannii* is rare in Turkey (between 1% and 4%).[20-22] Colistin resistance in *Pseudomonas aeruginosa* has been reported as 3% in the literature.[23,24] Colistin resistance in *Klebsiella pneumoniae* has been reported as between 1.5% and 50% in nearly all continents.[25-27] Studies evaluating the risk factors for infection with colistin-resistant microorganisms or colonized patients are limited. In this multicenter study, we aimed to investigate the risk factors for infection caused by colistin-resistant microorganisms.

PATIENTS AND METHODS

In this retrospective study, we used a 1:2 case-control ratio for our study population. An e-mail including the title and purpose of the study and data collection requirements was sent to 1500 infectious disease specialists via a scientific and social web portal named "Infeksiyon Dunyasi (Infection World)" (*http://www.infeksiyondunyasi.org/*). Patients infected with colistin-resistant *A baumannii*, colistin-resistant *P aeruginosa* and colistin-resistant *K pneumoniae* within the last 3 years were included in the case group. The centers participating in this study were asked to use the National Healthcare Safety Network criteria for diagnosis.[28,29] Colonized patients were not included in the study. The following data on patients was collected: age, gender, diagnosis on admission, the department where they were treated (or the type of intensive care unit), comorbidities, invasive procedures and treatments, antibiotics used in the last three months and their groups, hospitalization or intensive care unit (ICU) stay in the last three months, infection site, dosage of colistin use, length of hospital stay before the isolation of the colistin-resistant microorganism, disease severity score, microbiological or clinical cure, and prognosis. In the identification of hospital-acquired infections, the criteria of the Centers for Disease Control and Prevention (CDC) and the National Health Standards Institutes (NHSI) were used.[28,29]

We requested that the same data for each case be collected from two control patients followed-up in the same period (±1 week) and in the same unit. The patients in the control group were either not infected or infected with a gram-negative colistin-susceptible microorganism or gram-positive microorganism. Patients in the control group were selected from cases consecutively admitted in that period (±1 week) and whose demographic data were same by gender and within ±5 years for age. Admission diagnosis/comorbidities and illness severity scores were required to be similar to the patient group . Patients colonised with colistin-resistant microorganisms were not included in the control group.

We required that colistin resistance identified by conventional methods be confirmed using the E test recommended by Clinical Laboraty Standard Instistute.[30] Microorganisms with a minimal inhibitory concentration of 4 µg/mL or more were considered colistin resistant.[31]

Statistical analysis

Data collected from twelve centers were combined and analyzed using SPSS 15.0 (SPSS, Chicago, IL, USA) program. In the analysis of categorical variables the chi-square test was used. In the analysis of continuous variables either the t test or the Mann Whitney U test were used. A *P* value less than .05 was considered statistically significant. Statistically significant variables in the univariate analysis were included in a stepwise backwards logistic regression analysis.

RESULTS

Twelve tertiary care centers, consisting of six university and six training and research hospitals, replied via email to the request for data. Eighteen infectious disease specialists responded. The analysis used data from 56 cases and 109 controls, after exclusion of 3 control patients that could not be matched to cases. Colistin-resistant microorganisms isolated were 29 *A baumannii* (51.8%), 18 *P aeruginosa* (32.1%) and 9 *Klebsiella spp*. No sta-

tistically significant difference was detected in the demographic characteristics and diagnosis on admission between the case and control groups (**Table 1**). In a univariate analysis for risk factors associated with colistin resistance, antibiotic use in the last three months (*P*=.003), colistin use in the last three months (*P*=.016), carbapenem use in the last three months (*P*=.027), quinolone use in the last three months (*P*=.001) and the length of hospital stay before the isolation of the multiresistant microorganism (*P*=.04) were statistically significant (**Table 2**).

A multivariate analysis that included these sig-

Table 1. Univariate analysis of demographic features and other risk factors associated with infection of colistin-resistant gram-negative microorganisms and prognosis.

Characteristics	Colistin S n=109 (%)	Colistin R n=56 (%)	*P*
Gender			.15
Male	69 (63.3)	30 (53.6)	
Female	40 (36.7)	26 (46.4)	
Age (mean)	62.2 (18.4)	58.2 (18.5)	.19
APACHE II (mean)	17.2 (6.2)	17.9 (9.6)	.39
Cerebral infarct or hemorrhage and other CNS[a] pathologies	17 (15.6)	10 (17.9)	.88
Hospitalization in the ICU[b]	87 (79.8)	43 (76.8)	.33
ICU type[c]			
Medical	27/87 (31.0)	12/44 (27.3)	.80
Medical/Surgical (Reanimation)	46/87 (52.9)	26/44 (59.1)	.75
Surgical	14/87 (16.1)	6/44 (13.6)	.78
Comorbidity			
Yes	81 (74.3)	42 (75.0)	.54
No	28 (25.7)	14 (25.0)	
Diabetes mellitus	40 (36.7)	18 (32.1)	.68
Chronic renal failure	10 (9.2)	10 (17.9)	.08
COPD[d]	29 (26.6)	15 (26.8)	.56
Malignancy	24 (22.0)	12 (21.4)	.55
Heart failure	29 (26.6)	18 (32.1)	.46
Immunosuppression	6 (5.5)	6 (1.7)	.37
Surgery	39 (35.8)	22 (39.3)	.39
Hospitalization (last 3 months)	44 (40.4)	25 (44.6)	.36
Hospitalization in the ICU (last 3 months)	18 (16.5)	15 (26.8)	.36
VAP[e]	76 (69.7)	32 (57.1)	.08
Colistin dosage[f]			.43
3×75mg IV	14/40 (35.0)	10/33 (30.3)	
2×150 mg or 3×100 mg IV	26/40 (65.0)	23/33 (69.7)	
Exitus[g]	49(45.0)	30 (53.6)	.29

[a]CNS: Central nervous system; [b]ICU: Intensive care unit; [c]Each unit was compared other two units; [d]COPD: Chronic obstructive pulmonary disease; [e]VAP: Ventilatory-associated pneumoniae; [f]The patients have chronical renal insufficiency were excluded from the analysis; [g]Crude mortality.

nificant parameters showed that quinolone use in the last 3 months increased the risk of infection 3.2 times (*P*=.003, 95% CI:1.50-6.74) and colistin use in the last three months increased risk 3.6 times (*P*<.001, 95% CI:1.63-7.99). Further subgroup analysis by microorganisms could not be performed because of low numbers.

DISCUSSION

An increase in carbapenem resistance in multi-resistant microorganisms such as *A baumannii*, *K pneumoniae* and *P aeruginosa* has led to the increased use of colistin.[2] As an inevitable outcome of increased colistin use, colistin-resistant strains have developed through selection of heteroresistant strains.[1,2] Colistin has been widely used in Turkey since 2010. Colistin resistance is threatening as it can be related to a poor prognosis.[32] Not clarifying an optimal dosage and therefore using colistin in different dosages may induce inappropriate usage, the emergence of resistance and its continuous spread.[33]

Published reports on risk factors for colistin resistance are limited.[1,3,32] Matthaiou et al reported in 2008 on 41 cases including 35 infected and 6 colonized with colistin-resistant *K pneumoniae* (33), *Acinetobacter spp* (6), and *P aeruginosa* (2). In a univariate analysis, colistin use, duration of previous colistin use, patient age, history of previously performed surgical procedures, duration of stay in the ICU, monobactam use and duration of antifungal agent use were reported as risk factors. In the multivariate analysis of the same study, only colistin use was determined to be statistically significant and an independent risk factor.[3] In colistin resistance, the selection of strains plays a role. In another study, in colistin-susceptible Acinetobacter types isolated from the patient group using colistin, heteroresistant strains were shown to be significantly higher than in Acinetobacter types isolated from the patient group not using colistin.[34] Colistin heteroresistance was defined as an isolate with colistin an MIC of 2 mg/L, in which detectable subpopulations were able to grow in the presence of >2 mg/L colistin.[33] In *K pneumoniae* types resistant to carbapenem, the existence of heteroresistant strains has been shown.[35] In the multivariate analysis of our study, colistin use in the last three months was found to be a risk factor for colistin resistance. This points to the possibility that heteroresistant strains were selected by selective depression. However, a limitation of our study was in not performing a heteroresistance analysis on the strains. A heteroresistance analysis might have identified selection of heteroresistant strains as an important mechanism of colistin resistance in our study. In the multivariate analysis, another parameter found

Table 2. Univariate analysis of risk factors as invasive devices and antibiotic usage associated with infection of colistin-resistant gram-negative microorganisms and prognosis.

Invasive devices and treatment	Colistin S n=109 (%)	Colistin R n=56 (%)	*P*
Mechanical ventilation	71 (65.1)	39 (69.6)	.56
Central venous catheter	79 (72.5)	40 (71.4)	.89
Urinary catheter	91 (83.5)	45 (80.4)	.38
Steroid use	18 (16.5)	14 (25.0)	.21
Hemodialysis	13 (12.0)	12 (21.4)	.09
H2 receptor blocker or proton pump inhibitor use	89 (81.7)	48 (85.7)	.33
Total parenteral nutrition	55 (50.5)	30 (53.6)	.70
Nasogastric entubation	67 (61.5)	36 (64.3)	.43
Previous antibiotic use (last 3 months)	73 (67.0)	49 (87.5)	.003
Colistin	16(14.7)	17 (30.4)	.016
Carbapenem	38 (34.9)	29 (51.8)	.027
Glycopeptide	25 (22.9)	11 (19.6)	.39
Cephalosporin	39 (35.8)	25 (44.6)	.17
Aminoglycoside	7 (6.4)	4 (7.1)	1.00
Quinolone	19 (17.4)	24 (42.9)	.001
Sulbactam	24 (22.0)	17 (30.4)	.16
Tigecycline	10 (9.2)	13 (23.2)	.15
Piperacillin-tazobactam	32 (29.4)	22 (39.3)	.13
Metronidazole	18 (16.5)	8 (14.3)	.45
Linezolide/daptomycine	21 (19.3)	18 (32.1)	.051
Anti-fungal agent	19 (17.4)	12 (21.4)	.33
Clinical cure	63(57.8)	27 (48.2)	.24
Microbiological cure	61 (65.6)	29 (51.8)	.07
Length of hospital stay before MDR infection (median, minimum-maximum)	14 (3-148)	21 (3-118)	.04
Length of ICU stay before MDR infection[a] (median, minimum-maximum)	14 (3-148)	24 (5-118)	.04

[a]MDR: multidrug resistant; Contributors: Husnu Pullukçu, Meltem Tasbakan, Ozlem Tunccan, Yasemin Tezer, Mehmet Ozden, Ozge Turhan, Rahmet Guner, Yasemin Cag, Fatma Bozkurt, Fatma Yılmaz Karadag, Elif Doyuk Kartal, Gokhan Gozel, Cemal Bulut, F. Şebnem Erdinç, Cibali Acikgoz, Mehmet A. Tasyaran.

to be significant was quinolone use in the last three months.

In Matthaiou's study, no significant difference in mortality was reported between the group infected and/or colonized with colistin-resistant and colistin-susceptible microorganisms.[3] In our study, mortality rates were higher in infected patients, but the difference was not statistically significant (53.6% to 45%) (**Table 1**).

Colistin resistance in *A baumannii* is thought to be related to insufficient dosage.[9] In our study, there was no significant difference in two different dosages used in patients infected with colistin-resistant gram-negative microorganisms (3×75 mg to 2×150/3×100 mg). A loading dose of colistin might be useful in critical patients,[36] and most experts think that colistin should be used in combination antibiotic therapy to prevent colistin resistance.[37,38] However, our study was limited in that we were unable to collect relevant data relating to the loading dose or the use of colistin in combination. We could only find one case-control study in medical literature that investigated risk factors for colistin resistance in *P aeruginosa*. According to the results of this study, risk factors in terms of pandrug-resistant *P aeruginosa* were reported as combined use of carbapenem for over 20 days, colistin combination use for more than 13 days, and more than 78 open suctioning procedures.[7] The global spread of resistant *K pneumoniae* and the limited number of treatment options have given rise to substantial concerns. Although results are contradictory, some studies suggest there is a relationship between colistin use and carbapenem-resistant *K pneumoniae*.[6,32,39] Colistin resistance can be seen in *K pneumoniae* and it has been reported that the resistance develops during treatment.[40,41] In a case-control study published by Zarkotou et al, risk factors for colistin-resistant carbapenamase producing *K pneumoniae* included referral to the study center from another hospital and long term use of beta-lactam/beta-lactamase inhibitor combination (18.7 [6.5] days to 10.5 [5.2] days, *P*=.002). Thirteen patients, of whom 8 were infected and 5 colonized with colistin-resistant strain, were included in the study as the case group. Although there was a greater ratio of colistin use in the patient group infected or colonized with colistin-resistant *K pneumoniae* (30.8% to 20.5%), no significant difference was detected. The duration of colistin use was not associated with colistin resistance. However, in the same study the authors speculated that the effect of colistin use could be underestimated due clonal transfer identified by molecular analysis.[32] In the univariate analysis of a study from Greece,[1] colistin treatment, the average stay in the ICU and the length of colistin treatment were significant variables. In the multivariate analysis, colistin treatment was a statistically significant risk factor. Ninety percent of the patients who were colonized with colistin-resistant *K pneumoniae* had been treated with colistin. This rate was reported as 56% for the control group.[1] Previous colistin use in the last three months was a risk factor in our study, but we could not perform subgroup analysis for each microorganism because of low numbers. In an epidemic of colistin-resistant *K pneumoniae*,[39] the cases were older than the controls and imipenem MIC levels were higher. Mortality and length of hospital stay were higher in the case group, but the difference was not statistically significant. In another recent study, colistin-resistance was independently associated with a poor prognosis in infections due to carbapenem-resistant *K pneumoniae*.[42] When evaluating all microorganisms concomitantly for colistin resistance, quinolone use and receiving colistin were found to be significant risk factors.

We conclude that limiting the use of quinolone and being aware that resistance can develop during colistin use are important.

Funding
No funding.

Transparency declaration
No conflict of interest.

REFERENCES

1. Kontopidou F, Plachouras D, Papadomichelakis E, Koukos G, Galani I, Poulakou G, et al. Colonization and infection by colistin-resistant gram-negative bacteria in a cohort of critically ill patients. Clin Microbiol Infect 2011;17:9-11.
2. Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections. Lancet Infect Dis 2006;6:589-601.
3. Matthaiou DK, Michalopoulos A, Rafailidis PI, Karageorgopoulos DE, Papaioannou V, Ntani G, et al. Risk factors associated with the isolation of colistin-resistant gram-negative bacteria: a matched case-control study. Crit Care Med 2008;36:807-11.
4. Landman D, Bratu S, Alam M, Quale J. Citywide emergence of *Pseudomonas aeruginosa* strains with reduced susceptibility to polymyxin B. J Antimicrob Chemother 2005;55:954-7.
5. Falagas ME, Bliziotis IA, Kasiakou SK Samonis G, Athanassopoulou P, Michalopoulos A. Outcome of infections due to pandrugresistant (PDR) gram-negative bacteria. BMC Infect Dis 2005;5:24.
6. Antoniadou A, Kontopidou F, Poulakou G, Koratzanis E, Galani I, Papadomichelakis E, et al. Colistin resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: First report of a multiclonal cluster. J Antimicrob Chemother 2007;59:786-90.
7. Mentzelopoulos SD, Pratikaki M, Platsouka E, Kraniotaki H, Zervakis D, Koutsoukou A et al. Prolonged use of carbapenems and colistin predisposes to ventilator-associated pneumonia by pandrug-resistant *Pseudomonas aeruginosa*. Intensive Care Med 2007;33:1524-32.
8. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis 2005;40:1333–41.
9. Lim LM, Ly N, Anderson D, Macander L, Jarkowski A 3rd, Forrest A, et al. Resurgence of colistin: A review of resistance, toxicity, pharmacodynamics and dosing. Pharmacotherapy 2010;30:1279-91.
10. Perez JC, Groisman EA. Acid pH activation of the PmrA/PmrB two-component regulatory system of *Salmonella enterica*. Mol Microbiol 2007;63:283–93.
11. Gunn JS, Miller SI. PhoP-PhoQ activates transcription of pmrAB, encoding a two-component regulatory system involved in *Salmonella typhimurium* antimicrobial peptide resistance. J Bacteriol 1996;178:6857–64.
12. Cai Y, Chai D, Wang R et al. Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 2012; 67: 1607-15.
13. Hejnar P, Kolar M, Hajek V, Liang B, Bai N. Characteristics of *Acinetobacter* strains (phenotype classification, antibiotic susceptibility and production of beta-lactamases) isolated from hemocultures from the patients at the Teaching Hospital in Olomouc. Acta Univ Palacki Olomuc Fac Med 1999;142:73-7.
14. Gomez-Garces JL, Aracil B, Gil Y Burillo A. Susceptibility of 228 non-fermenting gram-negative rods to tigecycline and six other antimicrobial drugs. J Chemother 2009;21:267–71.
15. Mezzatesta ML, Trovato G, Gona F, Nicolosi VM, Nicolosi D, Carattoli A, et al. In vitro activity of tigecycline and comparators against carbapenem-susceptible and resistant *Acinetobacter baumannii* clinical isolates in Italy. Ann Clin Microbiol Antimicrob 2008;7:4.
16. Quinteira S, Grosso F, Ramos H, Peixe L. Molecular epidemiology of imipenem-resistant *Acinetobacter haemolyticus* and *Acinetobacter baumannii* isolates carrying plasmid-mediated OXA-40 from a Portuguese hospital. Antimicrob Agents Chemother 2007;51:3465–6.
17. Garcia-Penuela E, Aznar E, Alarcon T, López-Brea M. Susceptibility pattern of *Acinetobacter baumannii* clinical isolates in Madrid vs. Hong Kong. Rev Esp Quimioter 2006;19:45–50.
18. Dobrewski R1, Savov E, Bernards AT, van den Barselaar M, Nordmann P, van den Broek PJ, et al. Genotypic diversity and antibiotic susceptibility of *Acinetobacter baumannii* isolates in a Bulgarian hospital. Clin Microbiol Infect 2006;12:1135–7.
19. Arroyo LA, Garcia-Curiel A, Pachon-Ibanez ME, van den Barselaar M, Nordmann P, van den Broek PJ, et al. Reliability of the E-test method for detection of colistin resistance in clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005;43:903–5
20. Evren E, Göçmen JS, Demirbilek M, Eda Alıskan H. Imipenem, meropenem, colistin, amikacin and fosfomycin susceptibilities of multidrug resistant *Acinetobacter baumannii* strains isolated from various clinical specimens. Gazi Medical Journal 2013;24:1-4.
21. Kirisci O, Ozkaya E, Caliskan A Özden S, Alkıs Koçtürk S. Investigation of resistance profiles of *Acinetobacter* spp. isolated from clinical specimens. ANKEM Derg 2013;27:140-6.
22. Aygencel G, Dizbay M, Ciftci A, Turkoglu M. Colistin-Resistant *Acinetobacter baumannii* Isolated from Ventilator-Associated Pneumonia: A Case Report from Turkey. Turkish Journal of Intensive Care 2010;9:164-7.
23. Pitt TL, Sparrow M, Warner M, Stefanidou M. Survey of resistance of *Pseudomonas aeruginosa* from UK patients with cystic fibrosis to six commonly prescribed antimicrobial agents. Thorax 2003;58:794-6.
24. Schülin T. In vitro activity of the aerosolized agents colistin and tobramycinn and five intravenous agents against *Pseudomonas aeruginosa* isolated from cystic fibrosis patients in southwestern Germany. J Antimicrob Chemother 2002;49:403-6.
25. Gales AC, Jones RN, Sader HS. Contemporary activity of colistin and polymyxin B against a worldwide collection of gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006-09). J Antimicrob Chemother 2011;66:2070-4.
26. Strenger V, Gschliesser T, Grisold A, Zarfel G, Feierl G, et al. Orally administered colistin leads to colistin-resistant intestinal flora and fails to prevent faecal colonisation with extended-spectrum beta-lactamase-producing enterobacteria in hospitalised newborns. Int J Antimicrob Agents 2011;37:67-9.
27. Halaby T, Al Naiemi N, Kluytmans J, van der Palen J, Vandenbroucke-Grauls CM. Emergence of colistin resistance in Enterobacteriaceae after the introduction of selective digestive tract decontamination in an intensive care unit. Antimicrob Agents Chemother 2013;57:3224-9.
28. American Thoracic Society and Infectious Disease Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171:388-416.
29. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309-32.
30. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 21th Informational Supplement M100-S21. CLSI, Wayne, PA, USA, 2011.
31. Littlewood JM, Koch C, Lambert PA, Høiby N, Elborn JS, Conway SP, et al. A ten year review of colomycin. Respir Med 2000;94:632-40.
32. Zarkotou O, Pournaras S, Voulgari E, Chrysos G, Prekates A, Voutsinas D, et al. Risk factors and outcomes associated with acquisition of colistin-resistant KPC-producing *Klebsiella pneumoniae*: a matched case-control study. J Clin Microbiol 2010;48:2271-74.
33. Yau W, Owen RJ, Poudyal A, Bell JM, Turnidge JD, Yu HH, et al. Colistin hetero-resistance in multidrug-resistant *Acinetobacter baumannii* clinical isolates from the western Pacific region in the SENTRY antimicrobial surveillance programme. J Infect 2009;58:138-44.
34. Hawley JS, Murray CK, Jorgensen JH. Colistin heteroresistance in *Acinetobacter* and its association with previous colistin therapy. Antimicrob Agents Chemother 2008;52:351-2.
35. Meletis G, Tzampaz E, Sianou E, Tzavaras I, Sofianou D, et al. Colistin heteroresistance in carbapenemase-producing *Klebsiella pneumoniae*. J Antimicrob Chemother 2011;66:946-7.
36. Plachouras D, Karvanen M, Friberg LE, Papadomichelakis E, Antoniadou A, Tsangaris I, et al. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. Antimicrob Agents Chemother 2009;53:3430–6.
37. Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 2012;67:1607-15.
38. Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother 2011;55:3284–94.
39. Marchaim D, Chopra T, Pogue JM, Perez F, Hujer AM, Rudin S, et al. Outbreak

of colistin-resistant, carbapenem-resistant *Klebsiella pneumoniae* in Metropolitan Detroit, Michigan. Antimicrob Agents Chemother 2011;55:593-9.

40. Elemam A, Rahimian J, Mandell W. Infection with panresistant *Klebsiella pneumoniae*: a report of 2 cases and a brief review of the literature. Clin Infect Dis 2009;49:271-74.

41. Lee J, Patel G, Huprikar S, Calfee DP, Jenkins SG. Decreased susceptibility of polymyxin B during treatment for carbapenem resistant *Klebsiella pneumoniae* infection. J Clin Microbiol 2009;47:1611-2.

42. Capone A, Giannella M, Fortini D, Giordano A, Meledandri M, Ballardini M et al. High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. Clin Microbiol Infect 2013;19:23-30.