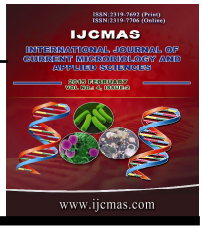
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 731-742

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 731-742**
http://www.ijcmas.com



Original Research Article

Antimicrobial activity of Colistin and Tiegecycline against carbapenem-resistant *Klebsiella pneumoniae* clinical isolates in Alexandria, Egypt

Sherine M.Shawky[1*], Amr Abdallah[2] and M.Khouly[3]

[1]Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria, Egypt
[2]Department of critical care medicine, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[3]Department of Microbiology and Immunology, Faculty of Pharmacy and Drug manufacturing, Pharos University, Alexandria, Egypt

**Corresponding author*

Keywords

Carbapenem-resistant, *K. pneumoniae* KPC, Carbapenemase, Colistin, Carbapenems, Tigecycline, Aminoglycosides

A B S T R A C T

The emergence of carbapemen resistant *K. pneumoniae* (CRKP) is becoming a significant health challenge. Infections caused by CRKP have limited treatment options and have been associated with high mortality rates. Our study aimed to investigate the antimicrobial susceptibility pattern, determine the antimicrobial activity of tigecycline and colistin as well as detecting the presence of colistin resistance among CRKP isolates in Alexandria, Egypt. This study included 139 CRKP isolates collected from different sites of infections**.** Identification and antibiotics susceptibility testing of isolates were performed using Vitek 2 compact system using GN identification card and GN 71 AST card respectively. Colistin MICs were determined by E-test. Our results were analyzed using CLSI 2014 and European Committee on Antibiotic Susceptibility Testing (EUCAST) breakpoints for *Enterobacteriaceae*. The present study showed that CRKP was most frequently isolated from blood stream infections (54 %) - in particular neonatal blood stream infections (29.4 %) - , followed by respiratory infections (22.3%), Wound/Surgical Operations (16.5%) and urine (7.2%). Our data had shown that high level of resistance existed among isolates against different classes of antibiotics; where all the isolates were resistant to all tested beta lactams and beta lactams / beta lactamase inibitor combination, 82 % of isolates were resistant to all tested fluroquinolones , while 57.5 % of isolates were resistant to all tested aminoglycosides, Among our CRKP isolates, 47.8 % were resistant to Trimethoprim / Sulphamethoxazole., while 17.3 % were resistant to Tigecycline. 65 isolates were randomly selected and tested against colistin out of which,9 isolates (13.8 %) were colistin resistant.

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a frequent nosocomial pathogen particularly in intensive care patients and among vulnerable individuals.(1) Most common Infections with *K. pneumoniae* are urinary and respiratory tract infections. *K.*

pneumoniae is a common cause of Gram-negative bloodstream infections especially in neonates.[2]

The emergence of strains of multidrug-resistant *K. pneumoniae* has been reported with increasing frequency in several countries worldwide.[3] During the past decades, the increased use of cephalosporins there has been accompanied by the emergence of *Enterobacteriaceae* possessing extended-spectrum blactamases (ESBLs).[4] WHO Reported resistance proportions to third generation cephalosporins in *K. pneumoniae* more than 30% resistance in the sampled populations.[1]

Carbapenems are broad spectrum antibiotics that are often used as last-resort treatments for resistant Gram negative infections caused by extended spectrum β-lactamase (ESBL) producing Enterobacteriaceae.[5] *K. pneumoniae* is the main cause of infections caused by carbapenem-resistant bacteria worldwide. In 2014, alarming rates of carbapenem resistance in *K. pneumonia* – exceeding 50% - have been reported in all WHO regions.[1] Infections caused by carbapenem-resistant *K.pneumoniae* (CRKP) are responsible for high morbidity and mortality rates. The mortality rates for *K. pneumoniae* hospital-acquired pneumonia exceed 50% in vulnerable patients.[1, 6]

Treatment of CRKP represents a major therapeutic challenge as most of the important genes that confer carbapenem resistance are present in *K. pneumonia* which renders limited number of agents available for treatment.[7]

Infections with carbapenem-resistant strains need to be treated with the last-resort drugs tigecycline or colistin, which clinicians are becoming increasingly dependent on for treatment of such infections.[1, 8]

Tigecycline is a minocycline derivative belonging to the new class of antimicrobials known as glycylcyclines. It is a broad-spectrum antimicrobial with activity against many Gram-positive, Gram-negative and anaerobic pathogens and has been frequently prescribed as a part of combination schemes against CR *Enterobacteriaceae*.[9-11] The main side effect of tigecycline is nausea. Other reported issues include pancreatitis and extreme alkaline phosphatase elevations. [12].

Polymyxins are the most common class of antibiotics used to treat carbapenem resistant Gram negative bacilli (CR GNB) as the cornerstone therapy.[13-17] Although resistance rates have been increasing in some countries, particularly among Enterobacteriaceae, polymyxins are still considered the most active agents against CR GNB.[18]

Polymyxins are a class of cyclic polypeptide antibiotics consisting of groups A-E, of which Polymyxin B and E (colistin) are currently available.[19] Colistin achieve concentration-dependent bactericidal killing and are often the only agents active against CRKP that achieve adequate levels in the serum to treat serious bloodstream infections.[20, 21]

In the past, Colistin was used infrequently, largely due to the associated nephrotoxicity and neurotoxicity, however the incidence of these adverse events does appear to be lower with modern preparations.[22]

Materials and Methods

The purpose of this study was to determine the antimicrobial activity of colistin and tigecycline against carbapenem-resistant *K. pneumonia*. Clinical isolates of CRKP were collected from patients in Alexandria main

university hospital, Queen Nazli Children hospital, and Mabaret EL Asafra hospital. Identification and antibiotics susceptibility testing of isolates were performed using Vitek 2 compact system (bio-Me´rieux, France) identification of isolates was done using GN identification cards.

Drug susceptibility testing (DST) for the *K. pneumoniae* strains was performed using the bioMe´rieux VITEK-2 AST-GN 71 system following manufacturer's instructions. The following 17 drugs were tested:

ampicillin (AMP), ampicillin/sulbactam (SAM), cefazolin (CFZ), ceftriaxone (CRO), cefepime (FEP), ertapenem (ETP), imipenem (IMP), meropenem (IMP), aztreonam (ATM), ciprofloxacin (CIP), Moxifloxacin (MXF), gentamicin (GM), tobramycin (TOB), amikacin (AMK), trimethoprim-sulfamethoxazole (SXT), Nitrofurantoin (FT) and Tigecycline (TGC). The ESBLs were also detected by the bioMe´rieux VITEK-2 AST-GN71 test.

Escherichia coli strains ATCC 25922 and ATCC 35218, *K. pneumoniae* ssp *pneumoniae* strain ATCC 700603 and *Pseudomonas aeruginosa* strain ATCC 27853 were used as quality control strains for the DST. Our results were analyzed using CLSI 2014 Carbapenem breakpoints for *Enterobacteriaceae*

Revised breakpoints MIC (µg/mL)

Table.1 New 2014 2014 CLSI revised breakpoints MIC (µg/mL) for *Enterobacteriaceae*

Agent	Susceptible	Intermediate	Resistant
Ertapenem	≤ 0.5	1	≥ 2
Imipenem	≤ 1	2	≥ 4
Meropenem	≤ 1	2	≥ 4

Tigecycline was evaluated using breakpoints for Enterobacteriaceae recommended by European Committee on Antibiotic Susceptibility Testing (EUCAST) ≤ 1, 2 and ≥ 4 µg/mL for susceptible, intermediate and resistant to tigecycline.

Colistin MICs were determined by E-test. For colistin the EUCAST clinical breakpoints for *Enterobacteriaceae* were applied (<= 2 µg/ml, susceptible; > 2 µg/ml, resistant). E-tests (AB Biodisk, Solna, Sweden) were used according to packet insert instructions, using Mueller-Hinton agar with an inoculum determined by a turbidity of 0.5 in a 0.85% NaCl suspension. The MIC endpoints were defined at the zone of complete inhibition of growth. Resistance to colistin was defined as an MIC of ≥ 4 µg /ml [(11)].

Result and Discussion

This study included 139 CRKP isolates collected from different sites of infections**)**: Blood 54 % (Neonates and Adults), Pulmonary 22.3 % (Sputum – MiniBAL and E.T.T), Wound/Surgical Operations 16.5 % (Pus Swabs – Wound Aspirates and Surgical drains) and Urine 7.2 % with the following distribution (Table 2).

All the isolates (100 %) were resistant to all tested beta lactams and beta lactams / beta lactamase inibitor combination : ampicillin (AMP), ampicillin/sulbactam (SAM), cefazolin (CFZ), ceftriaxone (CRO), cefepime (FEP), ertapenem (ETP), imipenem (IMP), meropenem (IMP) and aztreonam (ATM). The other tested antibiotics had the following susceptibility profile (Table 3)

For Aminoglycosides, high level of resistance was demonstrated among isolates against Tobramycin; only two isolates (1.44

%) were sensitive to the drug, whereas 28 % (39/139) and 22.3 % (31/139) were susceptible to Gentamicin and Amikacin respectively **(**Figure 1). Of importance to mention is that 29 (74.3 %) of Gentamicin susceptible isolates were collected from blood culture samples (n= 75), 75.86 % of which were isolated from neonates (22/29).

As regards, Susceptibility to fluroquinolones : 82 % of isolates were resistant to all tested fluroquinolones ; 17.2 % (24/139) of isolates were sensitive to moxifloxacin compared to 12.9 % (18/139) isolates were sensitive to Ciprofloxacin (Figure 2).

Among our CRKP isolates, 82.7 % (115/139) were sensitive to Tigecycline. Resistant strains (24 strains) 37.5 % (9/24) , 25 % (6/24) , 25 % (6/24) and 12.5 % (3/24) were isolated from pulmonary , urine , wound & surgical operations and blood cultures respectively while 48.2 % (67/139) of total isolates showed susceptibility to Trimethoprim / Sulphamethoxazole , 52.2 % (35/67) of these isolates were blood collected form blood culture samples (Figure 3).

Out of the 139 clinical isolates , 65 isolates were selected randomly according to frequency of samples type distribution to perform sensitivity testing against colistin using E-strip Test.

Nine CRKP of selected isolates were found to be colistin resistant representing 13.8 % (9 out of 65) isolated from the following sites of infections : 7 isolates from blood, 2 isolates from pulmonary and wound / surgical operations (Table 4).

The increasing spread of antimicrobial drug resistance among *K. pneumoniae* represents a significant clinical problem due to limited treatment options, as in addition to being resistant in vitro to all β-lactams and carbapenems, isolates are also frequently resistant to quinolones and aminoglycosides leaving the therapeutic options limited to tigecycline or colistin .[23]

However, reports of colistin- and tigecycline-resistant isolates have emerged; these isolates are commonly referred to as pan-resistant, owing to their resistance to all routine antibiotics. The spread of pan-resistant *K. pneumoniae* in acute care facilities could lead to significant morbidity and mortality.[24]
Unfortunately, the current situation of CRKP in Egypt has not been sufficiently studied, consequently, minimal data exist to guide selection of appropriate antimicrobial treatment regimen for CRKP isolates in Egypt.

In our study, CRKP was most frequently isolated from blood in particular neonatal blood stream infections which represent 29.5% of all samples, followed by respiratory (22.3%), Wound/Surgical Operations (16.5%), urine (7.2%).These findings were consistent with a case series review in which the most common site of infection caused by *K. pneumoniae* was blood (52%), followed by respiratory (30%), and urine (10%).[25]

Our results have shown that high level of resistance existed among isolates against different classes of antibiotics. Aminoglycoside resistance is increasing among CRKP bacteria. In the current study, 57.5 % of isolates were resistant to all tested aminoglycosides.

Data have shown rapid bactericidal activity of gentamicin against gentamicin-susceptible strains.[20] this finding was in consistence with our results as the highest sensitivity among the tested

aminoglycosides was associated with gentamicin (notably in neonatal blood stream infections) followed by 22.3 % in case of amikacin. These results were in agreement with Castanheira et al study in which 58.3 % of their resistant isolates were susceptible to gentamicin followed by amikacin 53.3 %. Of note, tobramycin, rarely displays in vitro activity against CRKP. This agrees with our finding as only two of our isolates were susceptible to tobramycin [26]

Despite its use in clinical practice for off-label indications, few studies have evaluated outcomes of serious MDR-*Enterobacteriaceae* infections treated with tigecycline, and even fewer have been dedicated to infections caused by CRKP bacteria. There are no breakpoints set by the CLSI for tigecycline for *Enterobacteriaceae* (FDA approved breakpoint for tigecycline is < 2 μg/mL). Tigecycline has demonstrated excellent spectrum of activity against MDR organisms. In a collection of 106 carbapenemase-producing strains from various countries, tigecycline was the only antimicrobial with 100% activity.[27] In the current study tigecycline showed satisfactory results as 82.7% of isolates were susceptible to the drug.

Also, in a review of 10 studies including 33 patients with infections caused by MDR-*Enterobacteriaceae*, Kelesidis *et al* reported favorable outcomes with tigecycline treatment in 70% of cases.34. 49% of these were cases of intra-abdominal infections, for which tigecycline has been approved.[28]

Development of resistance during tigecycline therapy has been described in several reports and remains a concern.[29, 30]. Between January and July of 2009 in one New York City hospital, 14 *K. pneumoniae* isolates were identified as intermediate or resistant to tigecycline.[31]

In an evaluation of 48 patients with carbapenem-resistant *K. pneumonia* bacteremia in a tertiary care hospital, all 8 patients with break-through bacteremias had received tigecycline [32] in the present study 17.3% of isolates were resistant to tigecycline

Clinical issues with tigecycline's pharmacokinetic properties may raise some caution in treatment of UTIs and BSIs due to low urinary (< 22%) and low plasma (≤ 0.9 mg/L) concentrations [33, 34]. In our study 60 % of our urinary klebsiella isolates were resistant to tigecycline,

Colistin, which had been in disuse for decades due to concerns about toxicity and availability of safer antimicrobial agents, now constitutes a first-line regimen for treatment of infection caused carbapenem-resistant *Enterobacteriaceae*.[17]

Additionally, no interpretative breakpoint has been defined by the Clinical and Laboratory Standards Institute (CLSI) [35]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has set the clinical breakpoint for *Enterobacteriaceae*, and resistance to colistin has been defined as a minimum inhibitory concentration (MIC) of 4 μg/mL.[36]

In most cases, colistin is the last viable effective option for the treatment of invasive bloodstream infections (BSI).[37] In the present study 86% of our colistin tested isolates were sensitive to the drug.

With the continued use of colistin for treatment of infection with various multidrug-resistant Gram-negative pathogens, t here are Increasing reports of polymyxin resistance. [17, 38-43]

Table.2 Distribution of CRKP bacterial isolates according to site of infections

Sample Type	No. (%)
I) Blood	75 (54 %)
a) Neonates	41 (29.4%)
b) Adults	34 (24.4%)
II) Pulmonary	31 (22.3%)
III) Wound / Surgical Operations	23 (16.5%)
IV) Urine	10 (7.2%)
Total	139

Table.3 Susceptibility profile of CRKP isolates to tested antibiotics

Antimicrobial Agent	Susceptibility No. (%)
I) Fluroquinolones	
Ciprofloxacin (CIP)	18 (12.9 %)
Moxifloxacin (MXF)	24 (17.2 %)
II) Aminoglycosides	
Tobramycin (TOB)	2 (1.44 %)
Gentamicin (GM)	39 (28 %)
Amikacin (AMK)	31 (22.3 %)
III) Trimethoprim-Sulfamethoxazole (SXT)	67 (48.2 %)
IV) Tetracycline	
Tigecycline (TGC)	115 (82.7 %)

Table.4 Distribution of the sire of infections and tigecycline susceptibility profile among the colistin resistant CRKP isolates

CRKP isolate number	Source of specimen	Colistin MIC	Tigecycline MIC
17	Abdominal drain	8	≥ 8 (R)
27	Blood culture	16	2 (S)
31	Blood culture	16	≥ 8 (R)
37	Blood culture	24	1 (S)
63	Blood culture	16	2 (S)
66	Blood culture	24	2 (S)
70	Blood culture	4	2 (S)
80	Blood culture	16	2 (S)
120	Sputum	4	2 (S)

S = Sensitive , R = Resistant

Figure.1 Aminoglycosides susceptibility pattern of CRKP isolates

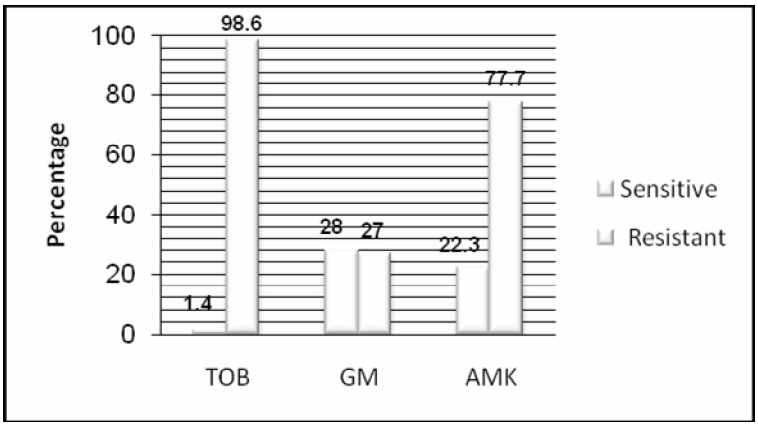


Figure.2 Fluroquinolones susceptibility pattern of CRKP isolates

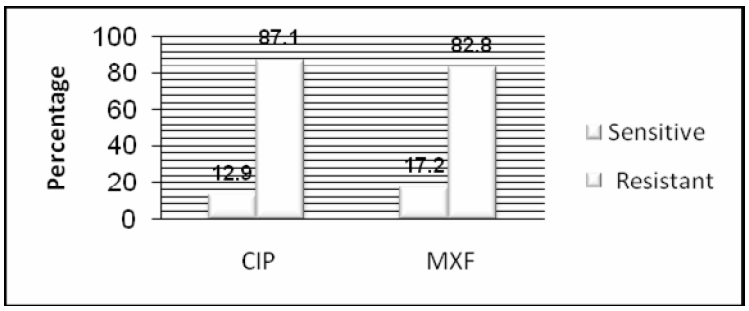


Figure.3 Trimethoprim / Sulphamethoxazole and Tigecycline susceptibility pattern of CRKP isolates

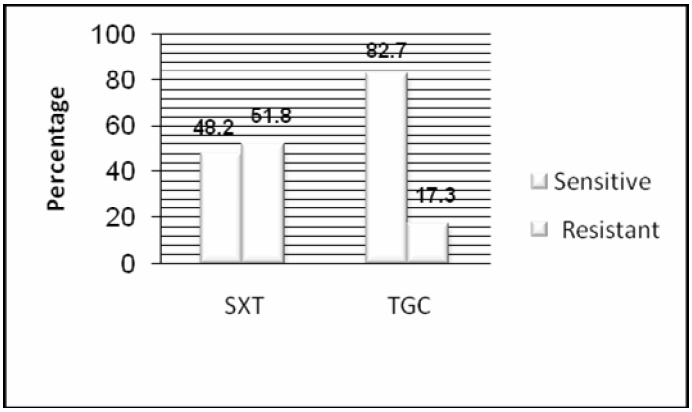


Figure.4 Colistin susceptibility pattern of CRKP isolates

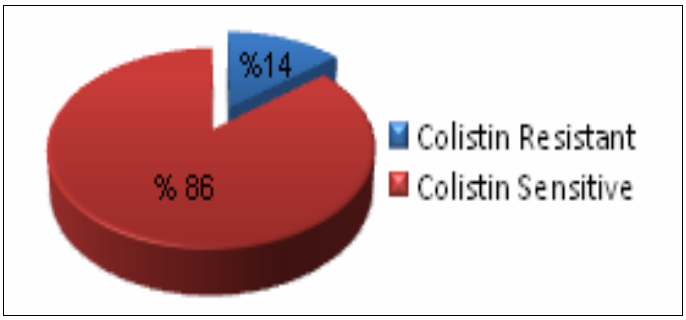


Figure.5 Colistin sensitive CRKP isolate (MIC = 0.5 µg/mL)

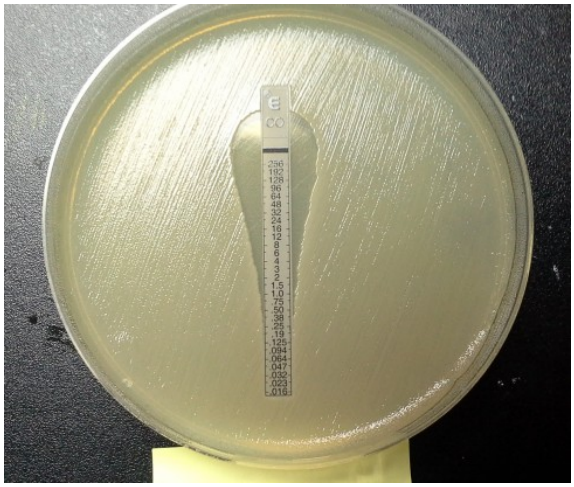
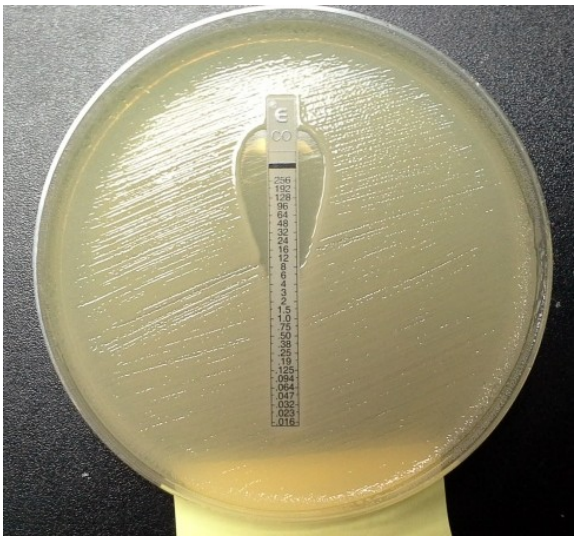


Figure.6 Colistin resistant CRKP isolate (MIC = 8 µg/mL)



In 2009, six *K. pneumoniae* were reported to be intermediate or resistant to polymyxin; two of these *K. pneumoniae* isolates were resistant to tigecycline, polymyxin and other antibiotics.[31] A major concern is that selective pressure due to extensive use or prior exposure to colistin, are likely to be of the driving factors fuelling the threat posed by colistin-resistant *Enterobacteriacae*.[8, 44-46]

In our study, 13.8 % (9/65) of the tested isolates were resistant to colistin , most of which were collected form blood stream infections (7/9). Infections caused by CRKP have been associated with high mortality rates and frequent treatment failure. Clinical data on treatment are limited and appropriate therapy for CRKP infections is not well defined.

Tigecycline is a bacteriostatic agent, serum and urinary levels of tigecycline are low, its use as monotherapy for blood stream or urinary tract infections needs to be further studied.

With the continued use of colistin for treatment of CRKP infection it is likely that there will be an increasing number of instances of both de novo emergence of resistance and nosocomial CRKP spread.

Combination therapy may be an important strategy for the management of CRKP infections when utilizing colistin. However, determining which antimicrobial in combination with colistin is superior still needs to be established clinical studies.

References

1. WHO. ANTIMICROBIAL RESISTANCE Global Report on surveillance 2014.
2. Lin YT, Wang FD, Chan YJ, Fu YC, Fung CP. Clinical and microbiological characteristics of tigecycline non-susceptible Klebsiella pneumoniae bacteremia in Taiwan. BMC Infect Dis2014;14:1.
3. Pereira GH, Garcia DO, Mostardeiro M, Fanti KS, Levin AS. Outbreak of carbapenem-resistant Klebsiella pneumoniae: two-year epidemiologic follow-up in a tertiary hospital. Mem Inst Oswaldo Cruz2013 Feb;108(1):113-5.
4. Zhang R, Yang L, Cai JC, Zhou HW, Chen GX. High-level carbapenem resistance in a Citrobacter freundii clinical isolate is due to a combination of KPC-2 production and decreased porin expression. J Med Microbiol2008 Mar;57(Pt 3):332-7.
5. Lee GC, Lawson KA, Burgess DS. Clinical epidemiology of carbapenem-resistant enterobacteriaceae in community hospitals: a case-case-control study. Ann Pharmacother2013 Sep;47(9):1115-21.
6. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in acute care facilities. MMWR Morb Mortal Wkly Rep2009 Mar 20;58(10):256-60.
7. Maltezou HC. Metallo-beta-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? Int J Antimicrob Agents2009 May;33(5):405 e1-7.
8. Lee J, Patel G, Huprikar S, Calfee DP, Jenkins SG. Decreased susceptibility to polymyxin B during treatment for carbapenem-resistant Klebsiella pneumoniae infection. J Clin Microbiol2009 May;47(5):1611-2.
9. Rose WE, Rybak MJ. Tigecycline: first of a new class of antimicrobial agents. Pharmacotherapy2006 Aug;26(8):1099-110.

10. Guner R, Hasanoglu I, Keske S, Kalem AK, Tasyaran MA. Outcomes in patients infected with carbapenem-resistant Acinetobacter baumannii and treated with tigecycline alone or in combination therapy. Infection2011 Dec;39(6):515-8.
11. Lee YT, Tsao SM, Hsueh PR. Clinical outcomes of tigecycline alone or in combination with other antimicrobial agents for the treatment of patients with healthcare-associated multidrug-resistant Acinetobacter baumannii infections. Eur J Clin Microbiol Infect Dis2013 Sep;32(9):1211-20.
12. Bonilla MF, Avery RK, Rehm SJ, Neuner EA, Isada CM, van Duin D. Extreme alkaline phosphatase elevation associated with tigecycline. J Antimicrob Chemother2011 Apr;66(4):952-3.
13. Capone A GM, Fortini D et al. High rate of colistin resistance among patients with carbapenem-resistant Klebsiella pneumoniae infection accounts for an excess of mortality. Clin Microbiol Infect2013;19(1):23–30.
14. Mammina C, Bonura C, Di Bernardo F, Aleo A, Fasciana T, Sodano C, Saporito MA, Verde MS, Tetamo R, Palma DM. Ongoing spread of colistin-resistant Klebsiella pneumoniae in different wards of an acute general hospital, Italy, June to December 2011. Euro Surveill2012;17(33).
15. Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother2012 Jul;67(7):1607-15.
16. Chen S, Hu F, Zhang X, Xu X, Liu Y, Zhu D, Wang H. Independent emergence of colistin-resistant Enterobacteriaceae clinical isolates without colistin treatment. J Clin Microbiol2011 Nov;49(11):4022-3.
17. Bogdanovich T, Adams-Haduch JM, Tian GB, Nguyen MH, Kwak EJ, Muto CA, Doi Y. Colistin-resistant, Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae belonging to the international epidemic clone ST258. Clin Infect Dis2011 Aug;53(4):373-6.
18. 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 Chemother2011 Sep;66(9):2070-4.
19. Giamarellou H, Poulakou G. Multidrug-resistant Gram-negative infections: what are the treatment options? Drugs2009 Oct 1;69(14):1879-901.
20. Bratu S, Tolaney P, Karumudi U, Quale J, Mooty M, Nichani S, Landman D. Carbapenemase-producing Klebsiella pneumoniae in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents. J Antimicrob Chemother2005 Jul;56(1):128-32.
21. Endimiani A, Hujer AM, Perez F, Bethel CR, Hujer KM, Kroeger J, Oethinger M, Paterson DL, Adams MD, Jacobs MR, Diekema DJ, Hall GS, Jenkins SG, Rice LB, Tenover FC, Bonomo RA. Characterization of blaKPC-containing Klebsiella pneumoniae isolates detected in different institutions in the Eastern USA. J Antimicrob Chemother2009 Mar;63(3):427-37.
22. Perez F, Van Duin D. Carbapenem-resistant Enterobacteriaceae: a menace to our most vulnerable patients. Cleve Clin J Med2013 Apr;80(4):225-33.

23. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis2009 Apr;9(4):228-36.
24. 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 Dis2009 Jul 15;49(2):271-4.
25. Lee GC, Burgess DS. Treatment of Klebsiella pneumoniae carbapenemase (KPC) infections: a review of published case series and case reports. Ann Clin Microbiol Antimicrob2012;11:32.
26. Satlin MJ, Kubin CJ, Blumenthal JS, Cohen AB, Furuya EY, Wilson SJ, Jenkins SG, Calfee DP. Comparative effectiveness of aminoglycosides, polymyxin B, and tigecycline for clearance of carbapenem-resistant Klebsiella pneumoniae from urine. Antimicrob Agents Chemother2011 Dec;55(12):5893-9.
27. Castanheira M, Sader HS, Deshpande LM, Fritsche TR, Jones RN. Antimicrobial activities of tigecycline and other broad-spectrum antimicrobials tested against serine carbapenemase- and metallo-beta-lactamase-producing Enterobacteriaceae: report from the SENTRY Antimicrobial Surveillance Program. Antimicrob Agents Chemother2008 Feb;52(2):570-3.
28. Kelesidis T, Karageorgopoulos DE, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant Enterobacteriaceae: a systematic review of the evidence from microbiological and clinical studies. J Antimicrob Chemother2008 Nov;62(5):895-904.
29. Anthony KB, Fishman NO, Linkin DR, Gasink LB, Edelstein PH, Lautenbach E. Clinical and microbiological outcomes of serious infections with multidrug-resistant gram-negative organisms treated with tigecycline. Clin Infect Dis2008 Feb 15;46(4):567-70.
30. Daly MW, Riddle DJ, Ledeboer NA, Dunne WM, Ritchie DJ. Tigecycline for treatment of pneumonia and empyema caused by carbapenemase-producing Klebsiella pneumoniae. Pharmacotherapy2007 Jul;27(7):1052-7.
31. Arnold RS, Thom KA, Sharma S, Phillips M, Johnson JK, Morgan DJ. Emergence of Klebsiella pneumoniae Carbapenemase (KPC)-producing bacteria. Southern medical journal2011;104(1):40.
32. Nguyen M, Eschenauer GA, Bryan M, O'Neil K, Furuya EY, Della-Latta P, Kubin CJ. Carbapenem-resistant Klebsiella pneumoniae bacteremia: factors correlated with clinical and microbiologic outcomes. Diagn Microbiol Infect Dis2010 Jun;67(2):180-4.
33. Peleg AY, Potoski BA, Rea R, Adams J, Sethi J, Capitano B, Husain S, Kwak EJ, Bhat SV, Paterson DL. Acinetobacter baumannii bloodstream infection while receiving tigecycline: a cautionary report. J Antimicrob Chemother2007 Jan;59(1):128-31.
34. Meagher AK, Ambrose PG, Grasela TH, Ellis-Grosse EJ. The pharmacokinetic and pharmacodynamic profile of tigecycline. Clin Infect Dis2005 Sep 1;41 Suppl 5:S333-40.
35. Clinical and Laboratory Standards Institute (2009) Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement. Approved standard M100-S19 Clinical and Laboratory Standards Institute, Wayne, PA.
36. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 20, valid from 2012-01-01.

37. Falagas ME, Fragoulis KN, Kasiakou SK, Sermaidis GJ, Michalopoulos A. Nephrotoxicity of intravenous colistin: a prospective evaluation. Int J Antimicrob Agents2005 Dec;26(6):504-7.
38. Matthaiou DK, Michalopoulos A, Rafailidis PI, Karageorgopoulos DE, Papaioannou V, Ntani G, Samonis G, Falagas ME. Risk factors associated with the isolation of colistin-resistant gram-negative bacteria: a matched case-control study. Crit Care Med2008 Mar;36(3):807-11.
39. Toth A, Damjanova I, Puskas E, Janvari L, Farkas M, Dobak A, Borocz K, Paszti J. Emergence of a colistin-resistant KPC-2-producing Klebsiella pneumoniae ST258 clone in Hungary. Eur J Clin Microbiol Infect Dis2010 Jul;29(7):765-9.
40. Zarkotou O, Pournaras S, Voulgari E, Chrysos G, Prekates A, Voutsinas D, Themeli-Digalaki K, Tsakris A. Risk factors and outcomes associated with acquisition of colistin-resistant KPC-producing Klebsiella pneumoniae: a matched case-control study. J Clin Microbiol2010 Jun;48(6):2271-4.
41. Humphries RM, Kelesidis T, Dien Bard J, Ward KW, Bhattacharya D, Lewinski MA. Successful treatment of pan-resistant Klebsiella pneumoniae pneumonia and bacteraemia with a combination of high-dose tigecycline and colistin. J Med Microbiol2010 Nov;59(Pt 11):1383-6.
42. Neonakis IK, Samonis G, Messaritakis H, Baritaki S, Georgiladakis A, Maraki S, Spandidos DA. Resistance status and evolution trends of Klebsiella pneumoniae isolates in a university hospital in Greece: ineffectiveness of carbapenems and increasing resistance to colistin. Chemotherapy2010; 56(6):448-52.
43. Jernigan MG, Press EG, Nguyen MH, Clancy CJ, Shields RK. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing Klebsiella pneumoniae. Antimicrobial agents and chemotherapy2012:AAC. 06364-11.
44. Falagas ME, Kasiakou SK, Kofteridis DP, Roditakis G, Samonis G. Effectiveness and nephrotoxicity of intravenous colistin for treatment of patients with infections due to polymyxin-only-susceptible (POS) gram-negative bacteria. Eur J Clin Microbiol Infect Dis2006 Sep;25(9):596-9.
45. Souli M, Galani I, Antoniadou A, Papadomichelakis E, Poulakou G, Panagea T, Vourli S, Zerva L, Armaganidis A, Kanellakopoulou K, Giamarellou H. An outbreak of infection due to beta-Lactamase Klebsiella pneumoniae Carbapenemase 2-producing K. pneumoniae in a Greek University Hospital: molecular characterization, epidemiology, and outcomes. Clin Infect Dis2010 Feb 1;50(3):364-73.
46. Garbati MA, Abdulhak AB, Baba K, Sakkijha H. Infection due to colistin-resistant Enterobacteriacae in critically-ill patients. The Journal of Infection in Developing Countries2013;7(10):713-9.