Original Research Article

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

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ABSTRACT

The emergence of carbapenem-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 fluoroquinolones, 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.

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

Introduction

Klebsiella pneumoniae (K. pneumoniae) is a frequent nosocomial pathogen particularly in intensive care patients and among vulnerable individuals. 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 has been accompanied by the emergence of *Enterobacteriaceae* possessing extended-spectrum β-lactamases (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
Identification and antibiotics susceptibility testing of isolates were performed using Vitek 2 compact system (bio-Mérieux, France) identification of isolates was done using GN identification cards.

Drug susceptibility testing (DST) for the *K. pneumoniae* strains was performed using the bioMérieux 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 bioMérieux 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>
<thead>
<tr>
<th>Agent</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertapenem</td>
<td>≤ 0.5</td>
<td>1</td>
<td>≥ 2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤ 1</td>
<td>2</td>
<td>≥ 4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤ 1</td>
<td>2</td>
<td>≥ 4</td>
</tr>
</tbody>
</table>

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.

**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 inhibitor 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 .

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. 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%), and 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%). 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. In 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-\textit{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 \textit{Enterobacteriaceae} (FDA approved breakpoint for tigecycline is \(< 2 \mu g/mL\)). 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-\textit{Enterobacteriaceae}, Kelesidis \textit{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 \textit{K. pneumoniae} isolates were identified as intermediate or resistant to tigecycline.\(^{(31)}\)

In an evaluation of 48 patients with carbapenem-resistant \textit{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 (\(< 0.9 \text{ mg/L}\)) 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 \textit{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 \textit{Enterobacteriaceae}, and resistance to colistin has been defined as a minimum inhibitory concentration (MIC) of 4 \text{\mu g/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,\(^{(17, 38-43)}\) there are Increasing reports of polymyxin resistance.
### Table 2 Distribution of CRKP bacterial isolates according to site of infections

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

### Table 3 Susceptibility profile of CRKP isolates to tested antibiotics

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Susceptibility No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I) Fluoroquinolones</strong></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>18 (12.9%)</td>
</tr>
<tr>
<td>Moxifloxacin (MXF)</td>
<td>24 (17.2%)</td>
</tr>
<tr>
<td><strong>II) Aminoglycosides</strong></td>
<td></td>
</tr>
<tr>
<td>Tobramycin (TOB)</td>
<td>2 (1.44%)</td>
</tr>
<tr>
<td>Gentamicin (GM)</td>
<td>39 (28%)</td>
</tr>
<tr>
<td>Amikacin (AMK)</td>
<td>31 (22.3%)</td>
</tr>
<tr>
<td><strong>III) Trimethoprim-Sulfamethoxazole (SXT)</strong></td>
<td>67 (48.2%)</td>
</tr>
<tr>
<td><strong>IV) Tetracycline</strong></td>
<td></td>
</tr>
<tr>
<td>Tigecycline (TGC)</td>
<td>115 (82.7%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>CRKP isolate number</th>
<th>Source of specimen</th>
<th>Colistin MIC</th>
<th>Tigecycline MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Abdominal drain</td>
<td>8</td>
<td>&gt; 8 (R)</td>
</tr>
<tr>
<td>27</td>
<td>Blood culture</td>
<td>16</td>
<td>2 (S)</td>
</tr>
<tr>
<td>31</td>
<td>Blood culture</td>
<td>16</td>
<td>&gt; 8 (R)</td>
</tr>
<tr>
<td>37</td>
<td>Blood culture</td>
<td>24</td>
<td>1 (S)</td>
</tr>
<tr>
<td>63</td>
<td>Blood culture</td>
<td>16</td>
<td>2 (S)</td>
</tr>
<tr>
<td>66</td>
<td>Blood culture</td>
<td>24</td>
<td>2 (S)</td>
</tr>
<tr>
<td>70</td>
<td>Blood culture</td>
<td>4</td>
<td>2 (S)</td>
</tr>
<tr>
<td>80</td>
<td>Blood culture</td>
<td>16</td>
<td>2 (S)</td>
</tr>
<tr>
<td>120</td>
<td>Sputum</td>
<td>4</td>
<td>2 (S)</td>
</tr>
</tbody>
</table>

S = Sensitive , R = Resistant
Figure 1 Aminoglycosides susceptibility pattern of CRKP isolates

![Aminoglycosides Susceptibility Pattern](image1)

Figure 2 Fluroquinolones susceptibility pattern of CRKP isolates

![Fluroquinolones Susceptibility Pattern](image2)

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

![Trimethoprim / Sulphamethoxazole and Tigecycline Susceptibility Pattern](image3)
**Figure 4** Colistin susceptibility pattern of CRKP isolates

![Pie chart showing colistin resistance and sensitivity]

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

![Image of colistin sensitive CRKP isolate]

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

![Image of colistin resistant CRKP isolate]
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 *Enterobacteriaceae*.\(^{8, 44-46}\)

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**


36. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 20, valid from 2012-01-01.


