

# Secular trends in *Klebsiella pneumoniae* isolated in a tertiary-care hospital: increasing prevalence and accelerated decline in antimicrobial susceptibility

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## ABSTRACT

**Introduction:** *Klebsiella pneumoniae* has become an increasingly important etiologic agent of nosocomial infections in recent years. This is mainly due to the expression of virulence factors and development of resistance to several antimicrobial drugs. **Methods:** This retrospective study examines data obtained from the microbiology laboratory of a Brazilian tertiary-care hospital. To assess temporal trends in prevalence and antimicrobial susceptibility, *K. pneumoniae* isolates were analyzed from 2000 to 2013. The relative frequencies of *K. pneumoniae* isolation were calculated among all Gram-negative bacilli isolated in each period analyzed. Susceptibility tests were performed using automated systems. **Results:** From 2000-2006, *K. pneumoniae* isolates comprised 10.7% of isolated Gram-negative bacilli (455/4260). From 2007-2013, this percentage was 18.1% (965/5331). Strictly considering isolates from bloodstream infections, the relative annual prevalence of *K. pneumoniae* increased from 14-17% to 27-32% during the same periods. A progressive decrease in *K. pneumoniae* susceptibility to all antimicrobial agents assessed was detected. Partial resistance was also observed to antimicrobial drugs that have been used more recently, such as colistin and tigecycline. **Conclusions:** Our study indicates that *K. pneumoniae* has become a major pathogen among hospitalized patients and confirms its recent trend of increasing antimicrobial resistance.

**Keywords:** *Klebsiella pneumoniae*. Antimicrobial resistance. Carbapenem. Amikacin. Secular trends.

## INTRODUCTION

In recent decades, *Klebsiella pneumoniae* has become an increasingly important nosocomial infectious agent, which is partly attributed to its increased expression of virulence factors that promote intra-hospital transmission and challenge infection control practices<sup>(1)</sup>. Additionally, strains with progressive reductions in antibacterial drug susceptibility have been isolated in different countries<sup>(2) (3)</sup>. Since the end of the 20<sup>th</sup> century, strains have been disseminated with simultaneous resistance to various antimicrobial classes mediated by the production of extended spectrum beta-lactamases (ESBL), loss of porins, and later also by the production of carbapenemases and metallo-beta-lactamases<sup>(4) (5)</sup>. Development of resistances to alternative drugs used to treat hospital-acquired *K. pneumoniae* infections, such as polymyxins and tigecycline, is currently being observed<sup>(6)</sup>.

Simultaneous and long-term analysis of *K. pneumoniae* susceptibility to different antimicrobials allows us to better

understand current and future perspectives for the therapeutic use of these drugs. This study aimed to describe secular trends of antimicrobial susceptibility among *K. pneumoniae* isolates over a 14-year period in a Brazilian tertiary-care hospital.

## METHODS

This is a retrospective study based on data obtained from the Microbiology Laboratory of the Ribeirão Preto Medical School Hospital at the University of São Paulo in Ribeirão Preto, Brazil. This is a public university-affiliated facility with approximately 800 active beds that provides tertiary acute care in many medical specialties for a reference population of around 3,000,000 persons. The study was performed from January 1, 2000 to December 31, 2013. During the entire study period, there was an antibiotic stewardship program actively promoted by the local Infection Control Service. This program includes weekly rounds performed by infectious disease specialists in critical areas, like intensive care units, and requires that all in-hospital antibiotic prescriptions have justification by the assistant physician of the patient, which is also audited by infectious diseases physicians. Those audits are specifically focused on the use of cephalosporins, carbapenems,  $\beta$ -lactamase inhibitor combinations, glycopeptides, aminoglycosides, quinolones, and polymyxins. A regularly updated guide for antimicrobial therapy

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is available for the prescribers in the hospital intranet. The Infection Control Service also promotes educational measures on the rational use of antibiotics and prevention of healthcare-associated infections.

We included all strains of *K. pneumoniae* isolated during the study period from any clinical specimens. To avoid the inclusion of duplicate strains, we considered only one isolate per patient in the same year. If a patient had a second infection episode apart from the first one, then, the second isolate counted as a new specimen. We did not differentiate between colonizing strains and truly infectious strains. We also did not examine if they were hospital-acquired or not. Although the vast majority of isolates included were considered hospital-acquired, a small portion of isolates was probably from community-acquired severe infections that led to the patient's admission.

We evaluated the relative frequency of *K. pneumoniae* isolation among all Gram-negative bacilli isolated in each analyzed period for all clinical specimens and specifically for blood cultures. The relative frequencies of *Escherichia coli* and *Pseudomonas aeruginosa* isolates in clinical samples were also estimated.

### Antimicrobial susceptibility tests

These tests were performed using the automated Microscan™ system (Siemens, USA) between 2000 and 2005 and by the automated Vitek™ system (Biomérieux, France) between 2006 and 2013.

Susceptibility tests were interpreted according to the recommendations of the Clinical Laboratory Standards Institute (CLSI, USA) over the study period<sup>(7)</sup>. The percentage of isolates susceptible to the assessed antibiotics was reported as a weighted mean for defined intervals.

### Aminoglycoside minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of amikacin and gentamicin were determined using the Vitek2™ system (Biomérieux) for *K. pneumoniae* isolated between January and June 2013. We selected 105 bacterial isolates obtained from blood, venous catheters, urine, surgical wounds, and deep abscesses. MIC distribution was compared between three bacterial phenotypes: a) carbapenem-resistant isolates, b) ESBL-producing carbapenem-susceptible isolates, and c) isolates with multiple susceptibilities.

### Ethical considerations

This study protocol was submitted to and approved by the institutional ethics review committee (number 8718/2015).

## RESULTS

During the period between 2000 and 2006, the mean number of Gram-negative bacilli isolated from hospitalized patients was 4,260 (range, 4,020-4,564). The mean number of *K. pneumoniae* isolates was 455 (range, 395-498), corresponding to 10.7% of the total Gram-negative bacilli isolated. During the period between 2007 and 2013, the annual mean numbers

of Gram-negative bacilli and of *K. pneumoniae* were 5,331 (range, 4,002-5,967) and 965 (range, 581-1,208), respectively, representing a relative frequency of 18.1% for *K. pneumoniae* during that period. **Figure 1** shows a gradual increase in *K. pneumoniae* isolation in relation to the total Gram-negative bacilli, particularly starting from 2006-2007. Comparatively, the relative percentage of *Escherichia coli* and *Pseudomonas aeruginosa* isolates remained stable. The relative frequency of *K. pneumoniae* compared to other Gram-negative bacilli causing bloodstream infections also increased from 14-17% between 2000 and 2003 to 27-32% between 2010 and 2013 (**Figure 1**).

Weighted mean *K. pneumoniae* rates of susceptibility to various classes of antimicrobials between 2000 and 2013 are presented in **Table 1**. Decreases in susceptibility to practically all antibiotics assessed were observed. Susceptibility to amikacin was maintained and even slightly increased from 2007 to 2013, exactly when a marked reduction in susceptibility to imipenem and ceftazidime occurred. Drugs that were more recently used in clinic, such as polymyxin and tigecycline, exhibited reduced *in vitro* activities against *K. pneumoniae* (**Figure 2**). Susceptibilities of 90% and 55% were observed to colistin and tigecycline, respectively, in 2013. The MIC for amikacin was similar between carbapenem-resistant and ESBL-producing *K. pneumoniae* isolates, but both were higher than that for multi-susceptible phenotypes (**Table 2**).

## DISCUSSION

The results of this study indicate a recent increase in *K. pneumoniae* colonization and infection of hospitalized patients, as well as a progressive reduction in the *in vitro* susceptibility of this *Enterobacteriaceae* to several antimicrobials<sup>(8)</sup>. The greater involvement of multidrug-resistant *K. pneumoniae* strains in hospital-acquired infectious has been simultaneously observed in Europe<sup>(9)</sup> and Asia<sup>(10)(11)</sup>. The expansion of *K. pneumoniae* as a colonizing and infectious agent in hospitals suggests a marked capacity of this bacterium to acquire resistance and survive in environments where antimicrobials are extensively used. A study conducted in the same hospital reported that *K. pneumoniae* strains expressed genes that increase resistance to carbapenems and fluoroquinolones as well as genes related to virulence factors<sup>(12)</sup>. Carbapenemase-producing *K. pneumoniae* that expresses genes related to capsule, fimbriae, siderophores and other virulence factors has been isolated in distinct geographic areas<sup>(1)(13)</sup>. There is evidence that *K. pneumoniae* has developed clones with multiple simultaneous resistance to antimicrobials and sufficient virulence to provide adaptive advantages to the hospital environment and facilitate patient colonization or infection<sup>(4)</sup>.

In the study institution, many antibiotics were introduced in the 1980s or 1990s. Since then, a continuous and variable reduction in *K. pneumoniae* susceptibility to these drugs has occurred. Long-term susceptibility studies have detected increasing antimicrobial drug resistance in hospitals in various countries<sup>(3)(14)</sup>. This is especially true for carbapenems. Few *K. pneumoniae* isolates were resistance to these antibiotics

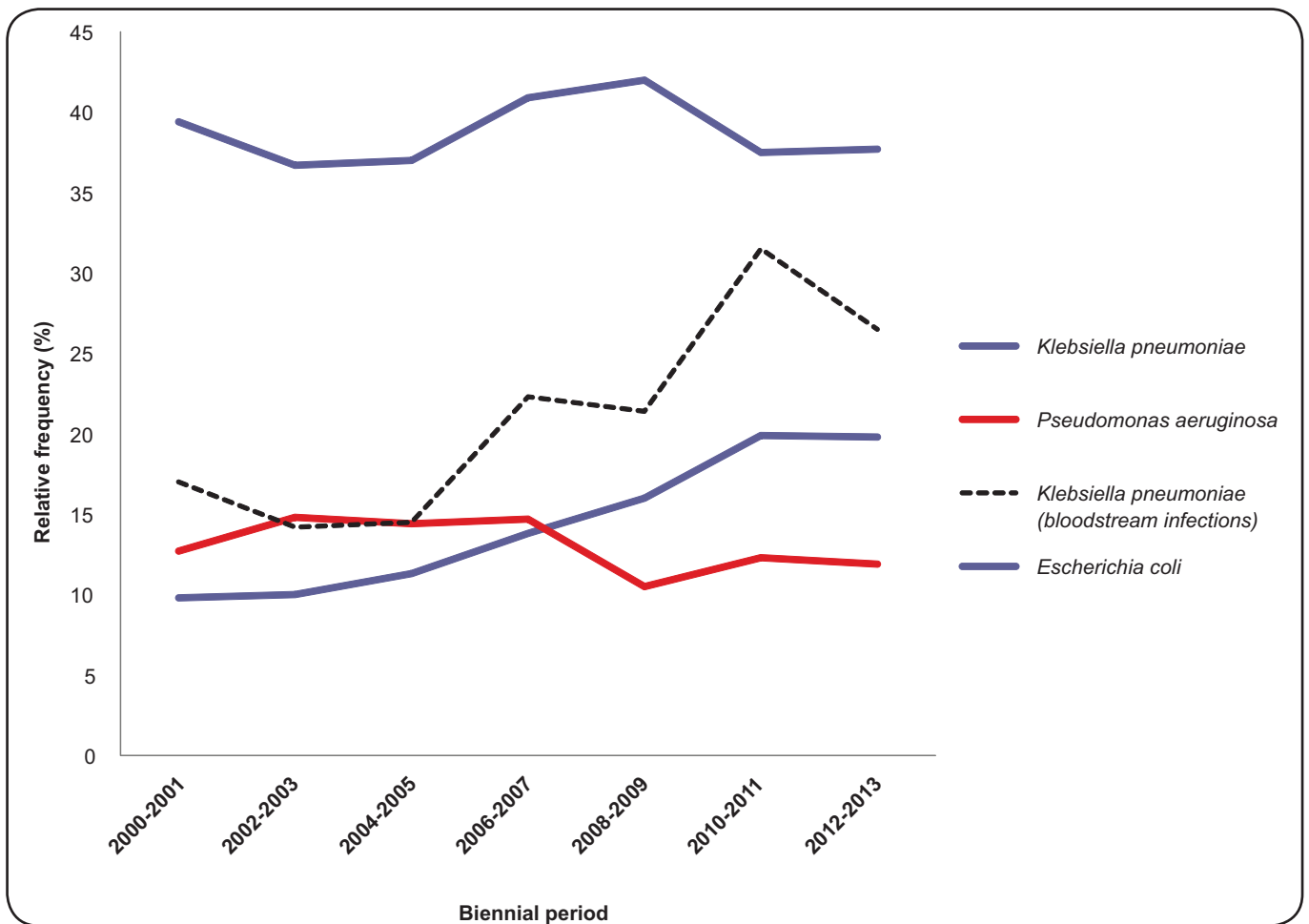


FIGURE 1 - Relative frequency of Gram-negative bacilli isolates for all specimens analyzed from 2000-2013. The dotted line represents the relative frequency specifically for bloodstream isolates.

up to 2007. Since 2008, carbapenem-resistant *K. pneumoniae* has spread to various wards of the hospital, and in 2012-2013, about 35% of the isolates had this phenotype. Between 2008 and 2013, a rapid reduction in susceptibility to carbapenems occurred together with an accelerated decrease in susceptibility to other beta-lactam antibiotics. Carbapenemase-producing *K. pneumoniae* have been noted in several regions in the world. In the USA, the isolation of carbapenem-resistant *K. pneumoniae* has increased from <0.1% in 2002 to 4.5% in 2010, while resistance to ceftazidime has increased from 5.3% in 1999 to 11.5% in 2010<sup>(15)</sup>. Data from the SENTRY study of intensive care units in Europe revealed a reduction in susceptibility to imipenem from 100% in 2009 to 89.7% in 2011<sup>(16)</sup>. A multicenter study showed that resistance to imipenem increased in Brazil in 2008-2010 (8.6%) compared to 2003-2005 (1.7%) and 1997-1999 (0.5%)<sup>(17)</sup>. A rapid increase in the rate of carbapenemase-producing *K. pneumoniae* isolation has been observed in Italian hospitals during a period similar to that in this study<sup>(18)</sup>. Currently, imipenem resistance rates may exceed 50%<sup>(2)</sup>.

The change made by CLSI in the carbapenem breakpoints for *Enterobacteriaceae*<sup>(7)</sup> may have additionally reduced the

rate of isolate susceptibility to these drugs, particularly for ertapenem<sup>(19)</sup> <sup>(20)</sup>. However, the major reason for the increase in *K. pneumoniae* resistance to carbapenems is probably the international dissemination of bacterial clones, such as the CC11 clonal complex, which express genes that regulate the carbapenemases, including *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase 48 (OXA-48), and New Delhi metallo- $\beta$ -lactamase (NDM)<sup>(21)</sup> <sup>(22)</sup>.

Amikacin was outstanding among antimicrobials because of its high and stable rates of *K. pneumoniae* susceptibility, which has allowed clinical use of this drug in infections caused by carbapenemase-producing isolates. High *in vitro* efficacy of amikacin has been observed in other studies<sup>(13)</sup> <sup>(16)</sup> and has been attributed to the limited clinical use of aminoglycosides over the last decades<sup>(23)</sup>. However, its higher MIC for carbapenem-resistant isolates suggests that these isolates are intermediately resistant to amikacin<sup>(24)</sup>.

Polymyxins and tigecycline are the major therapeutic options for treatment of infections caused by carbapenemase-producing *K. pneumoniae*<sup>(6)</sup>. Although polymyxins were reintroduced in clinical practice a few years ago to combat multi-resistant

TABLE 1 - *In vitro* susceptibility of *Klebsiella pneumoniae* isolates to selected antimicrobials.

Antimicrobial	Susceptibility of the isolates*					
	2000-2003		2004-2008		2009-2013	
	n	%	n	%	n	n
Amoxicillin clavulanate	1,270	79.0	1,943	71.0	2,521	54.0
Aztreonam	1,285	66.0	1,004	53.0	2,263	44.0
Piperacillin-tazobactam	665	74.0	1,703	71.0	4,120	59.0
Cefoxitin	1,820	84.0	567	89.0	3,584	65.0
Ceftriaxone	2,098	74.0	2,042	68.0	4,821	48.0
Ceftazidime	2,224	77.0	1,742	65.0	4,526	48.0
Cefepime	1,068	72.0	2,531	72.0	5,105	51.0
Imipenem	2,213	97.0	2,531	98.0	2,723	73.0
Meropenem	515	95.0	654	97.0	5,104	78.0
Ciprofloxacin	2,097	82.0	2,575	54.0	5,105	47.0
Levofloxacin	883	83.0	1,972	63.0	2,885	50.0
Amikacin	2,210	75.0	2,531	74.0	5,105	94.0
Gentamicin	2,239	72.0	2,570	64.0	5,104	64.0
TMP-SMX	2,240	53.0	2,570	47.0	3,462	41.0

TMP-SMX: trimethoprim-sulfamethoxazole. \*Weighted mean for the period.

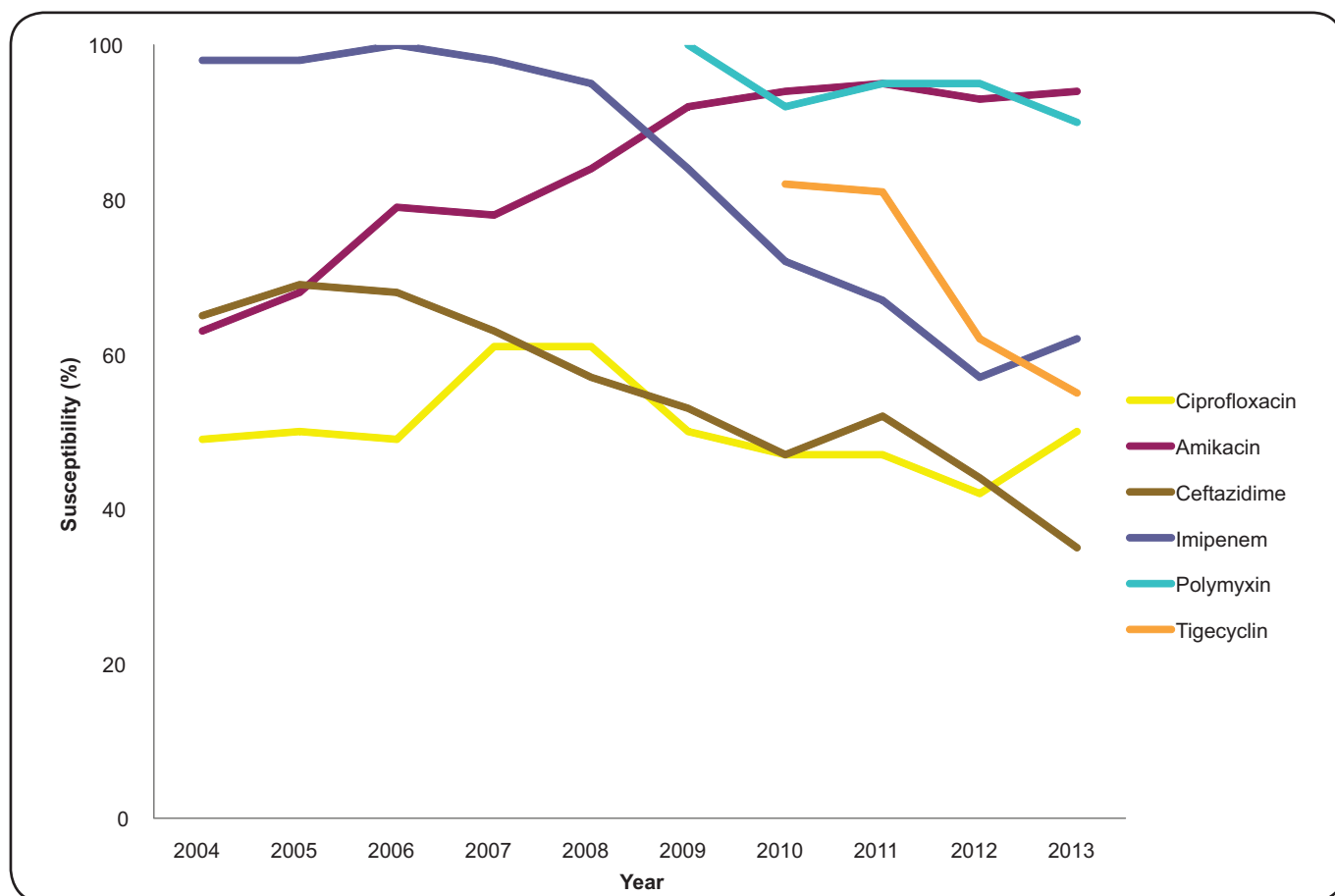


FIGURE 2 - Secular trends in antimicrobial susceptibility rates among *Klebsiella pneumoniae* isolates from 2004-2013.

**TABLE 2 - Minimum inhibitory concentrations of amikacin and gentamicin for *Klebsiella pneumoniae* isolates from nosocomial infection cases in 2013, according to ESBL or carbapenemase production.**

Antimicrobial	MIC (µg/mL)	<i>Klebsiella pneumoniae</i> phenotype		
		multi-susceptible (n=33)	ESBL-producing, carbapenem-susceptible (n=37)	carbapenem-resistant (n=35)
Amikacin	MIC50	≤2	4	≤2
	MIC90	≤2	16	16
	G-MIC	2	10.8	10.4
	S (%)	100	92	91
Gentamicin	MIC50	≤1	≥16	≥16
	MIC90	≤1	≥16	≥16
	G-MIC	1	9.9	12.4
	S (%)	100	35	23

ESBL: extended-spectrum β-lactamase; MIC: minimal inhibitory concentration; G-MIC: geometric mean minimal inhibitory concentration; S: susceptibility rate according to CLSI breakpoints; CLSI: Clinical and Laboratory Standards Institute.

Gram-negative bacilli, they have shown a slow and progressive reduction in activity against carbapenemase-producing *K. pneumoniae*, which in some studies has reached resistance rates that exceed 20%<sup>(25)</sup> <sup>(26)</sup>. Hetero-resistance to colistin, which is used more extensively, as well as the long-term therapy with polymyxins are factors favoring the selection of strains resistant to these drugs<sup>(27)</sup>. The reduction in susceptibility to tigecycline occurred rapidly considering the short time for which this drug was in clinical use. This finding has also been observed in other countries<sup>(25)</sup> <sup>(28)</sup>.

This study is limited by its retrospective nature. Thus, the influence of specific events, such as outbreaks, on antibiotic susceptibility could not be precisely defined. Otherwise, the long assessment period could dilute the influence of eventual outbreaks when taking into account the long-term antimicrobial susceptibility tendencies at the institution in this study. The same idea should be considered with regard to the different methods used to assess antibiotic susceptibility. In the last eight years, the automated Vitek™ system became the only method used, and the current trend in antibiotic susceptibility is evident. Moreover, throughout the period of analysis, susceptibility results followed CLSI recommendations. Additionally, over the last 10 years, medical cases that are more complex have been admitted to the hospital, which results in an increased need for microbiological cultures and antimicrobial prescriptions. This may have contributed to the selection of resistant microorganisms, but it does not explain the specific increase in the relative frequency of *K. pneumoniae* isolation. Although the institution has made efforts to promote the rational use of antibiotics and prevent bacterial dissemination, these measures proved to be not effective enough. The limited number of experts in antimicrobial use hampers the audit of prescriptions. We also suspect that non-compliance with the guide for antimicrobial use is a factor that contributes to bacterial resistance.

In conclusion, our study indicates that *K. pneumoniae* has become a major pathogen among hospitalized patients and

confirms its recent increase in antimicrobial resistance, a fact that will further complicate the clinical management of infected patients.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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