Infection and Drug Resistance

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ORIGINAL RESEARCH

Defining the potency of amikacin against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* derived from Chinese hospitals using CLSI and inhalation-based breakpoints

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Objectives: We report the in vitro activity of amikacin and comparators against Gram-negative bacteria collected from blood and respiratory specimens in China during a 1-year period between December 2015 and December 2016.

Materials and methods: Minimum inhibitory concentrations (MICs) were determined by agar dilution methods using Clinical and Laboratory Standards Institute (CLSI) guidelines, and susceptibility was assessed using CLSI breakpoints, except for tigecycline against Enterobacteriaceae. A pharmacodynamic threshold MIC ≤ 256 mg/L was also applied for amikacin since its inhalation formulation has demonstrated activity up to these MICs.

Results: For *Escherichia coli*, including extended-spectrum beta-lactamase (ESBL)-producing isolates (45.7% of population), amikacin demonstrated excellent activity (93.0%–94.7% susceptible) similar to tigecycline, piperacillin/tazobactam, and the carbapenems. Against *Klebsiella pneumoniae*, only tigecycline retained susceptibility >90%; amikacin inhibited 83.7% and 71.1% of the total and ESBL-producing (24.2%) populations at its breakpoint, respectively. Amikacin susceptibility against *Pseudomonas aeruginosa* was 91.1%, and only polymyxin B (100%) achieved higher susceptibility rates. Susceptibility declined to 80.9% and 54.5% against carbapenem- and multidrug-resistant (MDR) isolates, respectively. Finally, MDR was very common (84.0%) among *Acinetobacter baumannii*, with amikacin susceptibility at 30.5% for all isolates and 17.3% for MDR isolates. Since the majority of the amikacin-resistant isolates had amikacin MICs > 256 mg/L, the use of the inhalation pharmacodynamic threshold did not substantially improve the CLSI susceptible value.

Conclusion: Amikacin portrayed comparable or better susceptibility rates to most of the tested antibiotics against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* in China. As few isolates had MICs of 32–256 mg/L, use of the CLSI breakpoint and inhalation pharmacodynamic threshold yielded similar overall susceptibilities.

Keywords: Gram-negative, resistance, minimum inhibitory concentration, aminoglycoside

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Introduction

Gram-negative bacteria are among the most common causes of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP).[1–3] Among them, *Pseudomonas aeruginosa* is often the most common pathogen isolated, followed by Enterobacteriaceae including *Escherichia coli* and *Klebsiella pneumoniae*.[4] Although

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less common, HAP and VAP caused by *Acinetobacter baumannii* is among the most challenging Gram-negatives to treat due to high virulence, increasing resistance, and few available antibiotics with activity.[4,5] Collectively, these organisms account for nearly three quarters of HAP and VAP cases.[6]

The World Health Organization (WHO) lists antibiotic resistance as one of the most significant threats to global health, food security, and development today.[7] While Gram-negative resistance is a concern worldwide, certain countries have rates well above the average. Multidrug resistance in the Asia-Pacific region has risen at an alarming pace, with few new antimicrobials available to address this increase.[8] New antibiotics or novel technology to improve administration of available antibiotics are needed to address resistant organisms in HAP and VAP in the Asia-Pacific countries.

Amikacin Inhale (BAY41-6551, Bayer Pharma AG, Berlin, Germany) is a reformulated solution of amikacin combined with an inhalational drug delivery system that is currently in Phase III development as an adjunctive therapy for the treatment of Gram-negative pneumonia in intubated and mechanically ventilated patients with HAP or VAP.[9] When administered twice daily through the proprietary Pulmonary Drug Delivery System (PDDS), amikacin concentrations in the tracheal aspirates of mechanically ventilated patients were ≥ 6,400 mg/L (25 × 256 mg/L) in 50% of patients. Simulation of these exposures in the in vitro pharmacodynamic model showed bactericidal activity against most multidrug-resistant (MDR) *K. pneumoniae* and *P. aeruginosa* tested with amikacin minimum inhibitory concentrations (MICs) ≤ 256 mg/L.[10] Herein, the in vitro potency of amikacin was assessed using Clinical and Laboratory Standards Institute (CLSI) and inhalation-based breakpoints against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* derived from Chinese patients with infection sources (ie, blood and respiratory tract) typically yielding pathogens in HAP/VAP infection.

Materials and methods

Participating sites

Fourteen hospitals geographically dispersed throughout China provided clinical non-duplicate blood and respiratory isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* that were collected between December 23, 2015, and December 30, 2016. Organisms were identified at each participating center using standard methods and shipped to the central laboratory (Peking University People's Hospital, Beijing, China) for determination of MICs. The study was reviewed and approved by the Ethical Committees at Peking University People's Hospital and Hartford Hospital (Hartford, CT, USA). The Ethical Committees waived the need for informed consent as no private health information was collected.

Antibiotic susceptibility testing

Antibiotic MICs were determined for each isolate by agar dilution at the central lab according to CLSI guidelines (M07-A9).[11] The antimicrobials tested included meropenem (Sumitomo Pharmaceuticals Co., Osaka, Japan), imipenem (Sigma-Aldrich Co., St Louis, MO, USA), ertapenem (Sigma-Aldrich), ceftazidime (Sigma-Aldrich), cefotaxime (Sigma-Aldrich), ceftriaxone (Sigma-Aldrich), cefepime (Sigma-Aldrich), piperacillin/tazobactam (TZP) (Wyeth Pharmaceuticals, Collegeville, PA, USA), cefotaxime/clavulanic acid (Sigma-Aldrich), cefoxitin (Sigma-Aldrich), amikacin (Sigma-Aldrich), ceftazidime/clavulanic acid (Sigma-Aldrich), ciprofloxacin (Bayer AG, Leverkusen, Germany), levofloxacin (Bayer AG), minocycline (Sigma-Aldrich), polymyxin B (Sigma-Aldrich), and tigecycline (Wyeth Pharmaceuticals). The procedures for each set of tests were validated by determining the MICs for reference strains (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *E. coli* ATCC 35218), as recommended by CLSI.[12] The MIC_{50}, MIC_{90}, range, and percent susceptibility were calculated based on CLSI or US Food and Drug Administration (FDA) (for tigecycline only) susceptibility breakpoints.[12] Amikacin susceptibility was also assessed using a pharmacodynamic-based threshold MIC of ≤ 256 mg/L, which was derived from the in vitro pharmacodynamic model against *K. pneumoniae* and *P. aeruginosa*. When simulated at concentrations achievable in the epithelial lining fluid of patients receiving Amikacin Inhale (BAY41-6551), bactericidal activity was observed against isolates with MICs ≤ 256 mg/L.[10]

E. coli and *K. pneumoniae* were tested for extended-spectrum beta-lactamases (ESBL) production if they had an MIC of ≥ 1 mg/L against ceftazidime or cefotaxime. CLSI defined ESBL confirmation studies were undertaken using additional MIC testing of ceftazidime with clavulanic acid and cefotaxime with clavulanic acid. Enterobacteriaceae testing non-susceptible to ertapenem, meropenem, or imipenem were defined as carbapenem-resistant Enterobacteriaceae (CRE). Non-fermenters testing non-susceptible to meropenem or imipenem were defined as carbapenem-resistant *P. aeruginosa* (CRPA) or *A. baumannii* (CRAB), respectively.

The potency of amikacin was further assessed in organisms meeting the definition of MDR.[13] For the Enterobacteriaceae,

MDR was defined as an isolate non-susceptible to one agent in at least three of the following antimicrobial categories: aminoglycosides, piperacillin/tazobactam, carbapenems, 3rd or 4th generation cephalosporins, fluoroquinolones, glycylcyclines, polymyxins, or tetracyclines. For the *P. aeruginosa*, MDR was defined as an isolate non-susceptible to one agent in at least three of the following antimicrobial categories: aminoglycosides, piperacillin/tazobactam, group 2 carbapenems, anti-pseudomonal cephalosporins, fluoroquinolones, or polymyxins. For *A. baumannii*, MDR was defined as an isolate non-susceptible to one agent in at least three of the following antimicrobial categories: aminoglycosides, piperacillin/tazobactam, group 2 carbapenems, extended-spectrum cephalosporins, fluoroquinolones, polymyxins, or tetracyclines.

Results

Isolate numbers and patient characteristics

A total of 523 blood or respiratory isolates were tested as follows: *E. coli* (n = 151; all blood), *K. pneumoniae* (n = 129; blood, n = 120; respiratory, n = 9), *P. aeruginosa* (n = 112; blood, n = 57; respiratory, n = 55), and *A. baumannii* (n = 131; blood, n = 89; respiratory, n = 42). Patients contributing these samples were 58 ± 19 (range: 1–97) years old, and 207 (39.6%) were female.

E. coli and *K. pneumoniae*

Results for the *E. coli* and *K. pneumoniae* are provided in Table 1. Against *E. coli*, the most active antibiotics based on percent susceptibility included tigecycline (100% susceptible), the carbapenems (meropenem, imipenem, and ertapenem: 96.0%–98.0% susceptible), and amikacin (94.7% susceptible); 95.4% of *E. coli* had an MIC ≤ 256 mg/L. Polymyxin B also had potent activity against *E. coli*, but no CLSI or FDA breakpoints are currently identified for this agent. The ESBL rate among these *E. coli* was 86/188 (45.7%). Tigecycline, carbapenems, piperacillin/tazobactam, and amikacin retained susceptibility in the 90th percentile for ESBL-producing *E. coli*. Forty-four (29.1%) *E. coli* met the definition for an MDR organism. Amikacin MIC_{50}/MIC_{90} and percent susceptibility in these MDR organisms was 2/>256 mg/L and 81.8%, respectively; 84.1% of *E. coli* had an MIC ≤ 256 mg/L.

Against *K. pneumoniae*, the greatest susceptibility was observed for tigecycline (96.0%) followed by amikacin (83.7%), meropenem (82.2%), and ertapenem (80.6%). Polymyxin B displayed potent MICs as well similar to those against *E. coli*. The ESBL rate among these *K. pneumoniae* was 45/186 (24.2%). Only tigecycline susceptibility remained high at 95.6%, whereas amikacin declined to 71.1%. Carbapenems remained active against 77.8%–80.0% of ESBL-producing *K. pneumoniae*. Forty-four (34.1%) *K. pneumoniae* met the definition for an MDR organism. Amikacin MIC_{50}/MIC_{90} and percent susceptibility in these MDR organisms was 2/>256 mg/L and 63.6% (using both breakpoint and pharmacodynamic MIC threshold), respectively.

CRE was observed in 4.0% and 20.9% of *E. coli* and *K. pneumoniae* populations, respectively. Among the 33 CRE isolates, tigecycline provided the greatest susceptibility at 90.0%, followed by amikacin at 69.7% (using both thresholds). The polymxin B MIC_{50}/MIC_{90} was 0.25/0.25 mg/L. All other tested antibiotics portrayed susceptibility less than 30.3%.

P. aeruginosa and *A. baumannii*

P. aeruginosa and *A. baumannii* MIC and susceptibility results are provided in Table 2. Polymyxin B was the most active antibiotic against *P. aeruginosa* with an observed susceptibility of 100%, followed by amikacin at 91.1%, ciprofloxacin (83.9%), levofloxacin (81.3%), ceftazidime (81.3%), and cefepime (80.4%). Notably, carbapenem resistance was common in China with 47/112 (42%) isolates displaying non-susceptibility to either meropenem or imipenem. Among CRPA, polymyxin B susceptibility remained at 100%, while amikacin declined to 80.9%. Eighty-three percent of CRPA would be inhibited at ≤256 mg/L. Twenty-two (19.6%) *P. aeruginosa* met the definition for an MDR organism. Amikacin MIC_{50}/MIC_{90} and percent susceptibility in these MDR organisms was 8/>256 mg/L and 54.5%, respectively; 59.1% of *P. aeruginosa* had an MIC ≤ 256 mg/L.

For *A. baumannii*, the most active antibiotic was polymyxin B (99.2% susceptible) followed by minocycline (61.8%). Tigecycline displayed MICs that were 1–2 dilutions lower than minocycline, but no breakpoint is yet available for interpretation. Amikacin susceptibility was 30.5%, while susceptibility to the tested β-lactams and fluoroquinolones were less than 20%. CRAB was observed in the majority [111/131 (84.7%)] of isolates. Among CRAB, only polymyxin B retained reliable susceptibility at 99.1%. Amikacin susceptibility declined to 18.0%. Essentially all CRAB isolates (n = 110) met the definition for an MDR organism, with amikacin percent susceptibility at 17.3%; 18.2% of *A. baumannii* had an MIC ≤ 256 mg/L.

Table 1 MIC_{50}, MIC_{90}, range, and percent susceptibility for amikacin and comparators against *Escherichia coli* and *Klebsiella pneumoniae* isolates

Isolates	Antimicrobial	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	%S
E. coli, all isolates (n = 188)	Amikacin	2	4	1–>256	94.7 (95.4)[a]
	Cefoxitin	8	64	2–>256	62.9
	Cefepime	2	32	≤0.016–>256	53.6
	Ceftazidime	2	64	0.064–>256	64.9
	Cefotaxime	16	256	0.032–>256	32.5
	Ceftriaxone	32	256	0.032–>256	32.5
	Ciprofloxacin	16	>64	≤0.016–>64	34.4
	Ertapenem	0.032	0.25	≤0.016–>64	96.0
	Imipenem	0.125	0.25	≤0.016–16	98.0
	Levofloxacin	8	>64	≤0.016–>64	37.1
	Meropenem	≤0.016	0.032	≤0.016–>32	98.0
	Minocycline	2	16	0.5–128	72.8
	Piperacillin/tazobactam	2	8	0.5–>256	92.1
	Polymyxin B	0.125	0.25	0.064–8	NA[b]
	Tigecycline	0.25	0.5	0.125–2	100[c]
E. coli, ESBL producing (n = 86)	Amikacin	2	8	1–>256	93.0 (94.2)[a]
	Cefoxitin	8	32	2–>256	60.5
	Cefepime	8	32	0.032–>256	25.6
	Ceftazidime	4	64	0.125–>256	54.7
	Cefotaxime	64	256	2–>256	0.0
	Ceftriaxone	64	256	4–>256	0.0
	Ciprofloxacin	32	>64	≤0.016–>64	20.9
	Ertapenem	0.032	0.25	≤0.016–2	98.8
	Imipenem	0.125	0.25	≤0.016–0.5	100
	Levofloxacin	16	>64	≤0.016–>64	25.6
	Meropenem	≤0.016	0.032	≤0.016–0.125	100
	Minocycline	2	16	0.5–128	68.6
	Piperacillin/tazobactam	2	4	0.5–64	96.5
	Polymyxin B	0.125	0.25	0.064–8	NA[b]
	Tigecycline	0.25	0.5	0.125–2	100[c]
K. pneumoniae, all isolates (n = 186)	Amikacin	1	>256	0.5–>256	83.7 (83.7)[a]
	Cefoxitin	4	>256	1–>256	65.9
	Cefepime	0.25	64	≤0.016–>256	61.2
	Ceftazidime	0.5	128	0.032–>256	66.7
	Cefotaxime	0.5	256	≤0.016–>256	52.7
	Ceftriaxone	0.5	256	≤0.016–>256	51.2
	Ciprofloxacin	0.25	>64	≤0.016–>64	64.3
	Ertapenem	≤0.016	32	≤0.016–>32	80.6
	Imipenem	0.25	16	≤0.016–>32	79.8
	Levofloxacin	0.25	32	≤0.016–>64	69.0
	Meropenem	0.032	32	≤0.016–>32	82.2
	Minocycline	4	32	0.5–256	54.3
	Piperacillin/tazobactam	4	>256	≤0.016–>256	76.7
	Polymyxin B	0.25	0.5	0.064–4	NA[b]
	Tigecycline	1	2	≤0.016–8	96.1[c]
K. pneumoniae, ESBL producing (n = 45)	Amikacin	1	>256	0.5–>256	71.1 (71.1)[a]
	Cefoxitin	8	256	2–>256	60.0
	Cefepime	8	32	1–64	20.0
	Ceftazidime	16	64	0.25–>256	37.8
	Cefotaxime	64	256	4–>256	0.0
	Ceftriaxone	64	256	8–>256	0.0
	Ciprofloxacin	16	>64	0.032–>64	35.6
	Ertapenem	0.125	32	≤0.016–>32	77.8
	Imipenem	0.25	16	≤0.016–>32	77.8
	Levofloxacin	4	64	0.032–>64	44.4

(*Continued*)

Table 1 (*Continued*)

Isolates	Antimicrobial	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	%S
	Meropenem	0.032	8	≤0.016–>32	80.0
	Minocycline	8	64	0.5–128	26.7
	Piperacillin/tazobactam	4	>256	0.5–>256	71.1
	Polymyxin B	0.25	0.25	0.064–4	NA[b]
	Tigecycline	1	2	0.125–8	95.6[c]

Notes: [a]Amikacin percent susceptibility calculated using the CLSI breakpoint of ≤ 16 mg/L (PD threshold concentration: ≤ 256 mg/L). [b]Polymyxin B susceptibility breakpoints are not established by CLSI or the FDA. [c]Tigecycline percent susceptibility calculated using the FDA breakpoint of ≤ 2 mg/L.
Abbreviations: MIC, minimum inhibitory concentration; %S, percentage susceptibility; ESBL, extended-spectrum beta-lactamase; CLSI, Clinical and Laboratory Standards Institute; FDA, US Food and Drug Administration; PD, pharmacodynamic.

Table 2 MIC_{50}, MIC_{90}, range, and percent susceptibility for amikacin and comparators against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Isolates	Antimicrobial	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	%S
P. aeruginosa, all isolates (n = 112)	Amikacin	2	8	≤0.016–>256	91.1 (92.0)[a]
	Cefepime	2	16	≤0.016–256	80.4
	Ceftazidime	2	64	≤0.016–>256	81.3
	Ciprofloxacin	0.125	4	0.032–>64	83.9
	Imipenem	2	32	≤0.016–>32	59.8
	Levofloxacin	0.5	8	≤0.016–>64	81.3
	Meropenem	0.5	16	≤0.016–>32	68.8
	Piperacillin/tazobactam	4	128	≤0.016–>256	79.5
	Polymyxin B	0.5	1	0.032–2	100
P. aeruginosa, CRPA (n = 47)	Amikacin	2	>256	0.5–>256	80.9 (83.0)[a]
	Cefepime	8	64	1–256	59.6
	Ceftazidime	4	256	0.5–>256	66.0
	Ciprofloxacin	0.5	32	0.064–>64	68.1
	Imipenem	16	>32	2–>32	4.3
	Levofloxacin	2	32	0.125–>64	63.8
	Meropenem	8	>32	0.25–>32	25.5
	Piperacillin/tazobactam	16	256	0.25–>256	59.6
	Polymyxin B	0.5	1	0.25–2	100
A. baumannii, all isolates (n = 131)	Amikacin	>256	>256	1–>256	30.5 (31.3)[a]
	Cefepime	64	128	0.032–256	16.0
	Ceftazidime	128	>256	0.125–>256	18.3
	Ciprofloxacin	64	>64	≤0.016–>64	18.3
	Imipenem	>32	>32	0.25–>32	15.3
	Levofloxacin	8	16	0.032–32	19.1
	Meropenem	32	>32	0.032–>32	15.3
	Minocycline	4	16	0.032–256	61.8
	Piperacillin/tazobactam	>256	>256	≤0.016–>256	14.5
	Polymyxin B	0.25	0.5	0.125–>32	99.2
	Tigecycline	2	4	0.064–32	NA[b]
A. baumannii, CRAB (n = 111)	Amikacin	>256	>256	2–>256	18.0 (18.0)[a]
	Cefepime	64	128	2–256	0.9
	Ceftazidime	128	>256	4–>256	3.6
	Ciprofloxacin	64	>64	0.064–>64	3.6
	Imipenem	>32	>32	8–>32	0
	Levofloxacin	8	16	0.064–32	4.5
	Meropenem	32	>32	8–>32	0
	Minocycline	4	16	0.064–256	55.9
	Piperacillin/tazobactam	>256	>256	64–>256	0
	Polymyxin B	0.25	0.5	0.125–>32	99.1
	Tigecycline	2	4	0.125–32	NA[b]

Notes: [a]Amikacin percent susceptibility calculated using the CLSI breakpoint of ≤ 16 mg/L (PD threshold concentration: ≤ 256 mg/L). [b]Tigecycline susceptibility breakpoints are not established by CLSI or the FDA.
Abbreviations: MIC, minimum inhibitory concentration; %S, percentage susceptibility; CRPA, carbapenem-resistant *P. aeruginosa*; CRAB, carbapenem-resistant *A. baumannii*; CLSI, Clinical and Laboratory Standards Institute; FDA, US Food and Drug Administration; PD, pharmacodynamic.

Discussion

Amikacin Inhale provides a novel inhalation adjuvant therapy for the treatment of HAP and VAP by combining the potent in vitro activity of amikacin against Gram-negative bacteria and high concentrations achieved in the lower airways when administered via the proprietary PDDS.[9] In vitro pharmacodynamics studies have observed antibacterial activity up to and including MICs as high as 256 mg/L, four times the CLSI breakpoint.[10] The aim of this study was to compare the in vitro potency of amikacin with that of other intravenous antibiotics in China. We demonstrated widespread antimicrobial resistance in China among four common Gram-negative bacteria isolated from blood and respiratory tract samples. Notably, amikacin displayed good in vitro activity against most *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. In contrast, few antimicrobials had activity against the contemporary *A. baumannii* collected in China.

More than 90% of *E. coli* strains collected in this study were susceptible to amikacin, tigecycline, piperacillin/tazobactam, and the carbapenems. The 2015 Tigecycline Evaluation and Surveillance Trial (TEST) from the Asia-Pacific region observed similar susceptibility rates for these antibiotics with rates above 90%.[8] Amikacin susceptibility was specifically 98.1% in TEST versus 94.7% here. In contrast, ESBL rates among *E. coli* in these Chinese hospitals were more than twice that observed in the Asia-Pacific region as a whole (45.7% vs 21.6%). As a result, cephalosporin and fluoroquinolone susceptibility were observed to be lower herein. Among the ESBL-producing *E. coli*, amikacin, tigecycline, piperacillin/tazobactam, and the carbapenems retained excellent susceptibility rates, which is also congruent with TEST. It should be noted that polymyxin B MIC_{50} and MIC_{90} for these isolates were 0.125 and 0.25 mg/L, respectively. Although CLSI or FDA interpretative criteria for the polymyxins are not currently available for Enterobacteriaceae, MICs are well below the susceptibility breakpoints established for non-fermenting Gram-negative bacteria[12] and that of the colistin breakpoint (≤2 mg/L) for Enterobacteriaceae set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[14] While these in vitro data in and of themselves suggest that this antibiotic class may have a clinical role, it must be remembered that these antibacterials poorly penetrate the lung after intravenous administration thus their utility as monotherapy agents is severely limited in bronchopulmonary infection.

Against *K. pneumoniae* isolates, only tigecycline was observed to have susceptibilities above 90% in China, whereas amikacin, meropenen, and ertapenem demonstrated susceptibilities between 80.6% and 83.7%. These results again resemble observations from TEST from the Asia-Pacific region.[8] The ESBL rate among *K. pneumoniae* in China was 24.2% compared with 23.8% from the Asia-Pacific region as a whole; however, a closer evaluation of *K. pneumoniae* from China during 2004–2010 found a much higher ESBL rate of 63.6%. In contrast to the sustained susceptibility above 90% reported in TEST, amikacin susceptibilities against these ESBL-producing *K. pneumoniae* declined to 71.1% in China. As observed with *E. coli*, polymyxin B MICs typically remained below 0.5 mg/L against these *K. pneumoniae* isolates.

CRE was determined phenotypically in this study with rates of 4.0% and 20.9% among these *E. coli* and *K. pneumoniae*, respectively. These CRE demonstrated high rates of MDR as demonstrated by only tigecycline (90.0%) and amikacin (69.7%) having susceptibilities greater than ~30%. For amikacin, all isolates found to be non-susceptible had MICs >256 mg/L. The prevalence of the 16s rRNA methylase, the aminoglycoside modifying enzyme responsible for very high MICs (> 512 mg/L), has been reported to be endemic in regions of East Asia, including China.[15] This has potential clinical relevance since the Amikacin Inhale regimen has demonstrated antibacterial activity for isolates with MICs up to 256 mg/L, but not above this threshold. As a result of these high MICs among non-susceptible isolates, there was very little difference between amikacin susceptibility calculated using the CLSI breakpoint versus the in vitro model pharmacodynamic threshold. These observations in China are in contrast to ESBL-producing Enterobacteriaceae, for example, from the USA, in which 9% had MICs of 32 mg/L.[16]

Polymyxin B and amikacin were the only tested antibiotics demonstrating susceptibility greater than 90% against *P. aeruginosa*. Amikacin susceptibility in China (91.1%) was similar to rates reported in the Asia-Pacific region during TEST. (92.5%), as well as in the USA (95%).[8,16] Similar to the Enterobacteriaceae, few *P. aeruginosa* harbored amikacin MICs between 32 and 256 mg/L. Carbapenem resistance was common in *P. aeruginosa* (42.0%); against these isolates, amikacin susceptibility was 80.9%, and 83.0% of isolates were inhibited at 256 mg/L.

A. baumannii is a problematic isolate worldwide due to high levels of resistance secondary production of Class D carbapenemases, such as OXA-23, OXA-24 or -40, OXA-51, OXA-58, and OXA-143.[17] The isolates collected herein were no different with carbapenem resistance apparent in 84.7% of the population and MDR present in 84.0%. The majority of tested antibiotics (carbapenems, cephalosporins, and

fluoroquinolones) demonstrated susceptibility rates below 20%. Amikacin susceptibility was 30.5% and 17.3% for the total and MDR populations, respectively. Since the vast majority of the amikacin non-susceptible isolates by CLSI criteria had MICs > 256 mg/L, utilization of the inhalation breakpoint did very little to improve the overall susceptibility profile of amikacin against this Chinese population of *A. baumannii*. Only polymyxin B retained excellent susceptibility rates (99.1%–99.2%), followed by minocycline (55.9%–61.8%). Of note, tigecycline MIC_{50}/MIC_{90} was 2/4 mg/L, ~1–2 dilutions lower than minocycline; however, no interpretative susceptibility criteria are currently established for this agent. The rates of MDR *A. baumannii* were 48.5% in the Asia-Pacific region during the TEST surveillance study.[8] Similar poor susceptibilities were noted for all tested antibiotics, including amikacin, in that study as well.

Notable limitations of the current study include too few isolates within each species to conduct hospital-specific or regional distributional analyses. CLSI recommends no fewer than 30 isolates for an accurate identification of susceptibility, and splitting the current isolate collections by the 14 hospitals or the six representative regions would fall below that threshold. Additionally, only isolates collected from blood and respiratory tract specimens were included, as these are clinically pertinent to antibiotic used for pneumonia. Therefore, the observed resistance rates may not be representative or rates at other sources such as urine or wound, for example.

Conclusions

In summary, MDR resistance among *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* is common in China. Amikacin displayed similar, if not better, susceptibility rates versus the majority of available antibiotics, particularly against the *E. coli*, *K. pneumoniae*, and *P. aeruginosa* populations. However, few antibiotics, with the exception of polymyxin B, are available to address the MDR rates of *A. baumannii*. This study provides valuable data on the potential utility of Amikacin Inhale against Gram-negative bacteria from blood and respiratory tract sources in China.

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Disclosure

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