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Antimicrobial Susceptibility Testing for β-lactam/β-lactamase Inhibitor Combination Agents Against Enterobacterales Isolates Harboring β-lactamase Resistance Genes from North and Latin America: Comparison of VITEK® 2 AES and Broth Microdilution Methods

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Introduction

- β-Lactam agents are commonly used as the primary therapeutic options for serious infections caused by Enterobacterales isolates.
- Accurate susceptibility results for β-lactam/β-lactamase inhibitors (BL/BLIs) are crucial to treat Enterobacterales infections.
- The VITEK® 2 Advanced Expert System (AES) provides standardized phenotypic interpretation of MIC results based on an extensive database of MIC distributions and prevalent resistance mechanisms in Enterobacterales isolates.
- In this study, the BL/BLI susceptibility results from the VITEK® 2 AES were compared to the CLSI broth microdilution method (BMD) results against 513 molecularly characterized Enterobacterales isolates from North and Latin America.

Materials and Methods

Bacterial isolates

- A total of 407 clinical isolates were collected from 73 hospitals in 7 countries as part of the SENTRY Antimicrobial Surveillance Program during 2016–2019; an additional 106 isolates were from the CDC & US FDA Antibiotic Resistance Bank (Figure 1).
- Isolates were grouped into the following main group/species: *K. pneumoniae* (*n*=177), *E. coli* (*n*=134), *E. cloacae* species complex (*n*=72), and other Enterobacterales species (*n*=130; Figure 2A).

Susceptibility testing

- Broth microdilution (BMD) susceptibility testing for amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime-avibactam, ceftolozane-tazobactam, and meropenem-vaborbactam was conducted according to CLSI M07 guidelines using frozen-form, 96-well plates produced by JMI Laboratories.
- All isolates were tested by the VITEK® 2 with 9.02 software version using N802 and XN15 AST cards.
- MIC results were generated using the AES in the Global Clinical and Laboratory Studies Institute (CLSI)-based + Natural Resistance (NATR) mode and were reviewed by a microbiologist.
- CLSI clinical breakpoints were applied.
- Discordant results were repeated by both methods using the same inoculum.
- Essential (EA) and categorical agreement (CA) rates and error rates, such as very major (VME), major (ME), and minor error (mE) rates, were calculated based on the CLSI guidelines.

- Whole genome sequencing (WGS) was performed on isolates that met the following criteria by BMD:
  - *E. coli* and *K. pneumoniae* isolates displaying MIC values ≥2 mg/L for at least 2 of the following β-lactams: aztreonam, cefepime, ceftazidime, or ceftriaxone; and/or
  - Enterobacterales isolates displaying meropenem and/or imipenem MIC results >1 mg/L.
- Enterobacterales isolates that did not meet the criteria for molecular characterization were considered wildtype.

Results

- Among the isolates that met the molecular criteria, 211 harbored carbapenemase genes (41.1%), while 122 and 32 isolates carried ESBL (23.8%) and transferrable AmpC (6.2%) genes, respectively (Figure 2B).
- A total of 148 isolates were considered wildtype by acquired β-lactamase genes.
- Table 1 and Figures 3A and 3B display the VITEK® 2 EA and CA rates compared to BMD for each organism group.
- EA and CA rates were ≥90% for ampicillin-sulbactam, ceftazidime-avibactam, and meropenem-vaborbactam, except for *K. pneumoniae* (ceftazidime-avibactam EA, 85.3%) and other Enterobacterales (ampicillin-sulbactam CA, 84.0%).
  - Ampicillin-sulbactam displayed 1 VME and 7 mE against other Enterobacterales (Table 1).
- Amoxicillin-clavulanic acid EA rates were ≥90% for all organism groups, and CA rates were 89.3%, 88.8%, 98.6%, and 95.8% for *K. pneumoniae*, *E. coli*, *E. cloacae* complex, and other Enterobacterales, respectively (Table 1).
  - Amoxicillin-clavulanic acid discordances were mostly due to mE (35 occurrences) (Table 1).
  - Two VME and 3 ME were also noted (Table 1).
- Piperacillin-tazobactam EA rates were ≥90% for all organism groups, and CA rates were 93.2% (*K. pneumoniae*), 89.6% (*E. coli*), 87.3% (*E. cloacae* complex), and 89.4% (other Enterobacterales).
  - A total of 2 VME, 5 ME, and 38 mE were observed for piperacillin-tazobactam (Table 1).
- EA/CA rates were ≥90% for ceftolozane-tazobactam against *K. pneumoniae* and other Enterobacterales and were 89.6%/87.3% and 84.5%/87.3% against *E. coli* and *E. cloacae* complex, respectively.
  - Ceftolozane-tazobactam discordances were due to 1 VME, 12 ME, and 29 mE (Table 1).
- Table 2 displays the occurrence of VME and ME split by β-lactamase content.

Table 1. VITEK® 2 AES performance compared to BMD susceptibility testing

	No. of isolates	EA	CA	VME	%	ME	%	mE	%	R (BMD)	S (BMD)
Amoxicillin-Clavulanic acid											
Klebsiella pneumoniae	177	97.2%	89.3%	2	1.7%	1	2.3%	16	9.0%	117	43
Escherichia coli	134	99.3%	88.8%	0	0.0%	1	1.7%	14	10.4%	51	59
Enterobacter cloacae complex	72	98.6%	98.6%	0	0.0%	1	100.0%	0	0.0%	71	1
Other Enterobacterales	118	93.2%	95.8%	0	0.0%	0	0.0%	5	4.2%	76	36
Ampicillin-Sulbactam											
Klebsiella pneumoniae	177	99.4%	93.2%	1	0.7%	0	0.0%	11	6.2%	139	26
Escherichia coli	134	97.0%	91.0%	1	1.2%	2	5.7%	9	6.7%	82	35
Enterobacter cloacae complex[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Other Enterobacterales	50	96.0%	84.0%	1	3.8%	0	0.0%	7	14.0%	26	16
Ceftazidime-Avibactam											
Klebsiella pneumoniae	177	85.3%	98.9%	2	6.1%	0	0.0%	0	0.0%	33	144
Escherichia coli	134	97.0%	100.0%	0	0.0%	0	0.0%	0	0.0%	14	120
Enterobacter cloacae complex	71	93.0%	97.2%	0	0.0%	2	3.2%	0	0.0%	9	62
Other Enterobacterales	125	91.2%	98.4%	2	22.2%	0	0.0%	0	0.0%	9	116
Ceftolozane-Tazobactam											
Klebsiella pneumoniae	177	93.2%	94.4%	0	0.0%	7	11.3%	3	1.7%	111	62
Escherichia coli	134	89.6%	87.3%	0	0.0%	1	1.0%	16	11.9%	34	96
Enterobacter cloacae complex	71	84.5%	87.3%	1	2.2%	4	18.2%	4	5.6%	45	22
Other Enterobacterales	80	90.0%	92.5%	0	0.0%	0	0.0%	6	7.5%	20	55
Meropenem-Vaborbactam											
Klebsiella pneumoniae	177	90.4%	91.0%	1	2.9%	9	6.4%	6	3.4%	34	140
Escherichia coli	134	99.3%	100.0%	0	0.0%	0	0.0%	0	0.0%	14	120
Enterobacter cloacae complex	62	90.3%	95.2%	1	100.0%	0	0.0%	2	3.2%	1	59
Other Enterobacterales	124	98.4%	98.4%	0	NA	0	0.0%	2	1.6%	0	122
Piperacillin-Tazobactam											
Klebsiella pneumoniae	177	94.4%	93.2%	0	0.0%	1	1.7%	11	6.2%	113	58
Escherichia coli	134	91.8%	89.6%	0	0.0%	2	2.3%	12	9.0%	38	87
Enterobacter cloacae complex	71	93.0%	87.3%	0	0.0%	1	5.3%	8	11.3%	48	19
Other Enterobacterales	94	90.4%	89.4%	2	7.4%	1	1.6%	7	7.4%	27	61

Abbreviations: EA, essential agreement; CA, categorical agreement; VME, very major error; ME, major error; mE, minor error; R (BMD), resistance by broth microdilution; S (BMD), susceptible by broth microdilution.
[a] Ampicillin-sulbactam was not reported against *E. cloacae* complex due to intrinsic resistance.

Figure 1. Distribution of the Enterobacterales isolate collection (n=513) by country of origin or source

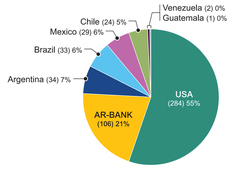


Figure 2. Characterization of Enterobacterales isolates (n=513) by species and β-lactam resistant genotype

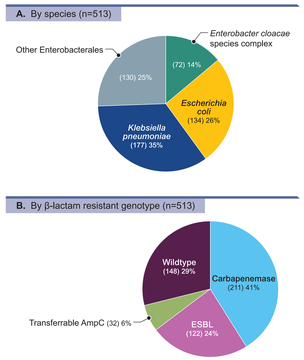
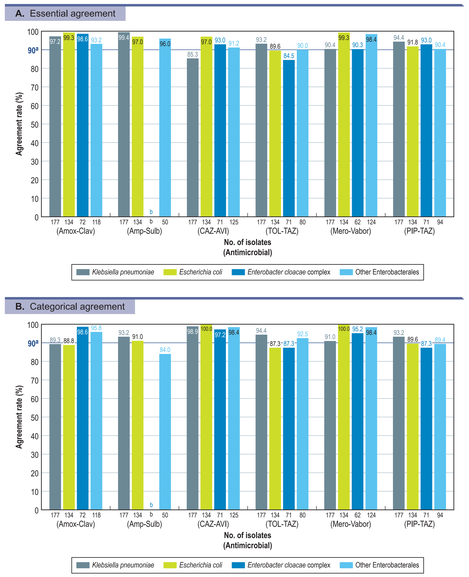


Figure 3. VITEK® 2 β-lactam/β-lactamase inhibitors EA and CA rates by Enterobacterales species



Abbreviations: Amox-Clav, amoxicillin-clavulanic acid; Amp-Sulb, ampicillin-sulbactam; CAZ-AVI, ceftazidime-avibactam; TOL-TAZ, ceftolozane-tazobactam; Mero-Vabor, meropenem-vaborbactam; PIP-TAZ, piperacillin-tazobactam.
[a] Horizontal blue line represents the 90% agreement cut-off.
[b] Ampicillin-sulbactam was not reported against *E. cloacae* complex due to intrinsic resistance.

Table 2. VME and ME occurrences by β-lactamase molecular category

	Carbapenemase		ESBL		tAmpC		Wildtype	
	ME	VME	ME	VME	ME	VME	ME	VME
Amoxicillin-Clavulanic acid	0	0	1	0	1	1	1	1
Ampicillin-Sulbactam	0	0	1	2	0	0	1	1
Ceftazidime-Avibactam	2	4	0	0	0	0	0	0
Ceftolozane-Tazobactam	1	1	8	0	0	0	3	0
Meropenem-Vaborbactam	5	2	2	0	1	0	1	0
Piperacillin-Tazobactam	1	2	2	0	1	0	1	0
Total	9	9	14	2	3	1	7	2

Abbreviations: tAmpC, transferrable AmpC; ME, major error; VME, very major error; ESBL, extended-spectrum β-lactamase.

Conclusions

- VITEK® 2 AES exhibited EA and CA rates ≥84% for 2 β-lactam/β-lactamase inhibitor agents against this challenge collection of Enterobacterales isolates carrying β-lactamase genes, regardless of species.
- Discordances mainly occurred due to minor errors.
- Clinicians and microbiologists should be aware that ME and VME may more frequently occur in some drug/bug combinations.

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