Plazomicin Susceptibility Testing using ETEST® MIC for Enterobacterales

Ying Y,[1] Armstrong T,[2] Blanchard L,[3] Denys G,[4] Halimi D,[5] Kresken M,[6] Pompilio M,[5] and Zambardi G[5]
[1]Quest Diagnostics, Lewisville, TX, USA; [2]bioMérieux Global Clinical Affairs, St Louis, MO, USA; [3]bioMérieux Global Clinical Affairs, Marcy-l'Étoile, France;
[4]Indiana University School of Medicine, Indianapolis, IN, USA; [5]bioMérieux, La Balme-les-Grottes, France; [6]Antiinfectives Intelligence GmbH, Reinbach, Germany.

IDWeek 2020
October 21-25, 2020

Correspondence: Yun (Claire) Ying
Yun.X.Ying@QuestDiagnostics.com

Introduction

- Plazomicin (PLZ, trade name ZEMDRI®) is an aminoglycoside antibiotic that has been shown to be active against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae*.
  - PLZ is indicated to treat complicated urinary tract infections (cUTI) in adults who have limited or no alternative treatment options.
- ETEST® (bioMérieux SA, Marcy-l'Étoile, France) is a manual, quantitative technique for determining antimicrobial susceptibility of nonfastidious Gram-negative and Gram-positive aerobic bacteria and fastidious bacteria (**Figure**).
  - The system comprises a predefined antibiotic gradient, which is used to determine the minimum inhibitory concentration (MIC, in µg/mL) of different antimicrobial agents against microorganisms tested on agar media after overnight incubation.[1]
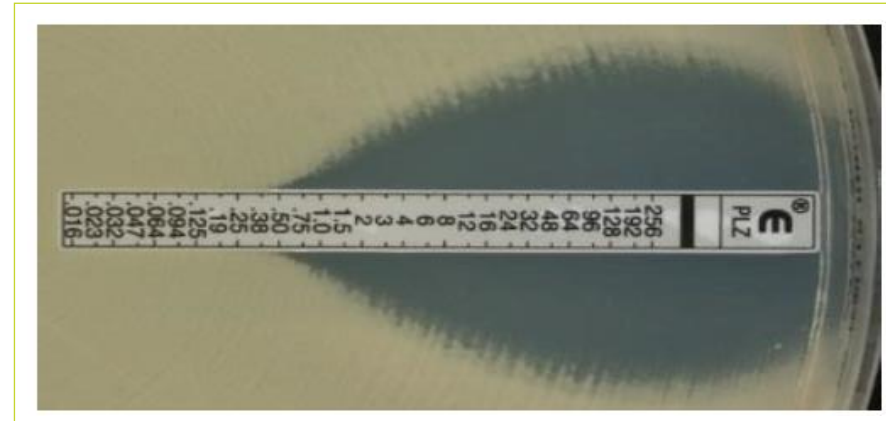

Figure. ETEST PLZ test for measuring PLZ MIC, here, on E Coli ATCC® 25922™.

- Here, we report the results of a clinical trial, which compared the performance of ETEST PLZ to the broth microdilution (BMD) reference method.

Methods

- The clinical trial was comprised 4 performance components, which were conducted at the following sites: bioMérieux SA Clinical Affairs Laboratory, Marcy-l'Étoile, France; Indiana University School of Medicine, Indianapolis, IN, USA; Quest Diagnostics at Medfusion, Lewisville, TX, USA; and Antiinfectives Intelligence GmbH, Cologne, Germany.
  - **(1 and 2) Clinical and challenge (comparative) studies:** The challenge component included 80 isolates; the clinical component included 518. The combined sets included 598 isolates of species listed in the US Food and Drug Administration (FDA)-approved drug instructions for use (IFU).[2]
  - **(3) Reproducibility study:** 10 isolates were tested in triplicate for 3 days at 3 clinical trial sites.
  - **(4) Quality control (QC):** QC was conducted following CLSI QC guidelines[3] using the following organisms: *Escherichia coli* ATCC 25922™, *Enterococcus faecalis* ATCC 29212™ on ETEST PLZ and BMD; Pseudomonas aeruginosa ATCC 27853™, and Staphylococcus aureus ATCC 29213™ on BMD only.
- Isolates were subcultured on tryptic soy or Columbia agar plates supplemented with 5% sheep blood before testing. After incubation, suspensions of the isolates were prepared in 0.85% saline solution. These suspensions were used to inoculate the BMD or Mueller Hinton agar for the ETEST. Results were read after 16 to 20 hours incubation at 35 ºC ±2 ºC in ambient air.
- Results were analyzed using US FDA breakpoints for PLZ: susceptible, ≤2 µg/mL; intermediate, 4 µg/mL; resistant ≥8 µg/mL.[4] There are no CLSI breakpoints to date.
- Performance was evaluated using US FDA performance criteria: essential agreement (EA) and category agreement (CA) ≥90%, major error rate ≤3.0% and very major error rate ≤2.0%.[5]

Methods (continued)

- **EA is defined as** a MIC result obtained with the antimicrobial susceptibility testing system (ASTS) within 1 doubling dilution step; **CA**, agreement of susceptible (S), intermediate (I), or resistant (R) between a MIC test and the reference method; **minor error**, difference in results between the ASTS and the reference method, one result being I and the other is susceptible or resistant; **major error**, reference method result is S and the ASTS result is R; **very major error**, reference method result is R and the ASTS result is S.[6]

Results

- A total of 598 isolates were tested in the comparative studies. The overall performance and MIC distribution are listed in **Tables 1 and 2**. Organisms that tested are listed in **Table 3**.
- Most clinical isolates were susceptible at 0.5 µg/mL PLZ (**Table 2**).

Table 1. Overall Performance of ETEST PLZ for Enterobacterales

EA, % (n)	CA, % (n)	Very major error rate, % (n)	Major error rate, % (n)	Minor error rate, % (n)
99.0 (592/598)	92.8 (555/598)	1.9 (1/53)	0.0 (0/478)	7.0 (42/598)

CA, categorical agreement; EA, essential agreement.

Table 2. Distribution of Test Performance and MICs of Isolates Tested With Each Testing System

ETEST PLZ MIC, µg/mL, and interpretation	Reference method MIC, µg/mL, and interpretation														Total, mode
	≤0.016 S	0.032 S	0.064 S	0.125 S	0.25 S	0.5 S	1 S	2 S	4 I	8 R	16 R	32 R	64 R	128 R	≥256 R	
<0.016 S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
0.016 S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
0.032 S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
0.064 S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
0.125 S	–	–	–	2	2	–	–	–	–	–	–	–	–	–	–	5
0.25 S	–	–	–	3	44	10	2	–	–	–	–	–	–	–	–	59
0.5 S	–	–	–	–	75	136	19	2	–	–	–	–	–	–	–	**232**
1 S	–	–	–	–	1	26	54	12	–	–	–	–	–	–	–	93
2 S	–	–	–	–	–	23	52	18	1	–	–	–	–	–	–	94
4 I	–	–	–	–	–	–	15	45	5	–	–	–	–	–	–	65
8 R	–	–	–	–	–	–	–	4	5	2	–	–	–	–	–	11
16 R	–	–	–	–	–	–	–	–	4	3	–	–	–	–	–	7
32 R	–	–	–	–	–	–	–	–	–	2	1	–	–	–	–	3
64 R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
128 R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
≥256 R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	29	30
Evaluable	–	–	–	5	122	172	98	81	67	15	7	1	–	–	–	
Total, mode	0	0	0	5	122	**172**	98	81	67	15	7	1	0	1	29	

CA, categorical agreement; EA, essential agreement; I, intermediate; MIC, minimum inhibitory concentration; S, susceptible; R, resistant.
Green indicates: EA, CA, and no error; **yellow:** CA, essential disagreement, and no error; **blue:** EA, categorical disagreement, and minor error; **red:** categorical and essential disagreement, and very major error.

Results (continued)

- ETEST PLZ performance was 100% in reproducibility (270/270) and QC studies (81/81 for each QC organism).

Table 3. Distribution of EA, CA, and Errors Among Organisms

Organism,	Study Component	EA (of total), n (%)	EA (of evaluable), n (%)	CA, n (%)	Resistant isolates, n	Very major error, n	Major error, n	Minor error, n
C freundii	Challenge	6/6 (100)	6/6 (100)	6/6 (100)	0	–	0	0
	Clinical	53/53 (100)	52/52 (100)	53/53 (100)	1	0	0	0
	Species total	59/59 (100)	58/58 (100)	59/59 (100)	1	0	0	0
C koseri	Challenge	3/3 (100)	3/3 (100)	3/3 (100)	0	–	0	0
	Clinical	30/31 (97)	30/31 (97)	31/31 (100)	0	0	0	0
	Species total	33/34 (97)	33/34 (97)	34/34 (100)	0	0	0	0
E cloacae	Challenge	10/10 (100)	10/10 (100)	10/10 (100)	0	–	0	0
	Clinical	50/50 (100)	48/48 (100)	50/50 (100)	2	0	0	0
	Species total	60/60 (100)	58/58 (100)	60/60 (100)	2	0	0	0
E coli	Challenge	13/13 (100)	8/8 (100)	10/13 (77)	5	0	0	3
	Clinical	63/65 (97)	62/64 (97)	65/65 (100)	1	0	0	0
	Species total	76/78 (97)	70/72 (97)	75/78 (96)	6	0	0	3
K aerogenes	Challenge	6/6 (100)	6/6 (100)	6/6 (100)	1	0	0	0
	Clinical	32/32 (100)	31/31 (100)	32/32 (100)	1	0	0	0
	Species total	38/38 (100)	37/37 (100)	38/38 (100)	2	0	0	0
K oxytoca	Challenge	7/7 (100)	7/7 (100)	6/7 (86)	0	–	0	1
	Clinical	32/32 (100)	31/31 (100)	31/32 (97)	1	0	0	1
	Species total	39/39 (100)	38/38 (100)	37/39 (95)	1	0	0	2
K pneumoniae	Challenge	17/17 (100)	13/13 (100)	16/17 (94)	7	0	0	1
	Clinical	72/72 (100)	63/63 (100)	72/72 (100)	11	0	0	0
	Species total	89/89 (100)	76/76 (100)	88/89 (99)	18	0	0	1
M morganii	Challenge	3/3 (100)	3/3 (100)	2/3 (67)	2	0	0	1
	Clinical	27/28 (96)	27/28 (96)	19/28 (68)	3	0	0	9
	Species total	30/31 (97)	30/31 (97)	21/31 (68)	5	0	0	10
P mirabilis	Challenge	4/4 (100)	4/4 (100)	4/4 (100)	2	0	0	0
	Clinical	59/59 (100)	57/57 (100)	50/59 (85)	6	0	0	9
	Species total	63/63 (100)	61/61 (100)	54/63 (86)	8	0	0	9
P stuartii	Challenge	2/2 (100)	1/1 (100)	2/2 (100)	1	0	0	0
	Clinical	33/33 (100)	30/30 (100)	24/33 (73)	7	0	0	9
	Species total	35/35 (100)	31/31 (100)	26/35 (74)	8	0	0	9
P vulgaris	Challenge	5/5 (100)	5/5 (100)	3/5 (60)	0	–	0	2
	Clinical	29/29 (100)	29/29 (100)	26/29 (90)	1	0	0	3
	Species total	34/34 (100)	34/34 (100)	29/34 (85)	1	0	0	5
S marcescens	Challenge	4/4 (100)	4/4 (100)	4/4 (100)	0	–	0	0
	Clinical	32/34 (94)	32/34 (94)	30/34 (88)	1	1	0	3
	Species total	36/38 (95)	36/38 (95)	34/38 (90)	1	1	0	3
Total	Challenge	80/80 (100)	70/70 (100)	72/80 (90)	18	0	0	8
	Clinical	512/518 (99)	492/498 (99)	483/518 (93)	35	1	0	34
Grand total		**592/598 (99)**	**562/568 (99)**	**555/598 (93)**	**53**	**1**	**0**	**42**

CA, categorical agreement; EA, essential agreement.
A dash (–) indicates very major error unable to be calculated owing to lack of resistant isolates.

Conclusions

- The clinical performance of the ETEST PLZ test met the US FDA acceptance criteria and was found useful for determining the MIC of Plazomicin for Enterobacterales, including those that produce extended spectrum beta-lactamase (ESBL) or high levels of AmpC β-lactamases; carbapenem-resistant Enterobacteriaceae (CRE, including those that produce metallo-β-lactamases [MBL], *K pneumoniae* carbapenemase [KPC], or oxacillinase-48 [OXA-48]); and aminoglycoside-resistant strains.
- Among the 598 isolates tested, the overall susceptibility to Plazomicin was 80%.
  - The mode MIC was low, at 0.5 µg/mL (susceptible).
- Plazomicin, as an aminoglycoside antibiotic, provides a treatment option for cUTI with multidrug-resistant Enterobacterales.
- The ETEST PLZ test is easy to use and has a quick turnaround time.

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Acknowledgments

Disclosure