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METHODOLOGY

Evaluation of Antimicrobial Susceptibility Testing Performance of DL 96 System for *Enterobacterales*

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Abstract: DL96 Microbial Identification/Antimicrobial Susceptibility Testing (ID/AST) System (Zhuhai DL, Guangdong, China) is one of the most commonly used commercial ID/AST System in China. This study aims to evaluate the performance of DL 96E for Antimicrobial Susceptibility Testing (AST) of 270 *Enterobacterales* isolates from Hainan general hospital using the broth microdilution method (BMD) as reference method. CLSI M52 criteria was followed when analyzing the evaluation results. Twenty antimicrobial agents were evaluated, and categorical agreement (CA) ranged from 62.8% to 96.5%. Imipenem had the lowest CA (63.9%) and highest very major errors (VME) (52.8%). A total of 103 carbapenem-resistant *Enterobacterales* were evaluated; DL 96E miss identified 22 isolates, including six carbapenemase-producing *Enterobacteriaceae*. DL 96E must adjust the Minimum Inhibitory Concentration (MIC) ranges of ciprofloxacin, levofloxacin, and piperacillin-tazobactam to cover Clinical and Laboratory Standards Institute (CLSI) breakpoints, adjust the formulation of some antimicrobial, such as imipenem, and increase the MIC detection range to cover the Quality control (QC) strains' MIC range.

Keywords: antimicrobial susceptibility testing, broth microdilution method, minimum inhibitory concentration, categorical agreement

Introduction

The DL 96 Microbial Identification/Antimicrobial Susceptibility Testing (ID/AST) System (Zhuhai DL, Guangdong, China) was launched in China 2003. Currently, there are more than 1000 institutions using DL 96 system in China and DL 96 is one of top three ID/AST systems in China. However, an investigation of China Antimicrobial Resistance Surveillance System (CARSS) indicates that most of DL users are Grade II hospitals that cannot verify the accuracy of ID/AST system after admission. DL 96 system has also been launched in many countries, including Southeast Asia, the Middle East, and Latin America. Till now, only two studies evaluating the accuracy of the DL 96 susceptibility were reported, one was for *Streptococcus*,[1] the other was for *Enterobacteriales*.[2] Dr. Xu compared five commonly used AST systems in the CARSS hospitals, including DL96.[2] The study found that DL 96 performed poorly in interpreting ESBL, imipenem, cephalosporin, and β-lactam combination agents. But this study only tested five strains, three were American Type Culture Collection (ATCC) strains, and two were "unknown" isolates. Therefore, more studies evaluating the accuracy of antimicrobial susceptibility testing to common bacteria are needed.

DL96 system has launched five different ID/AST combo kits, namely 96E for *Enterobacteriales*, 96NE for Gram-negative non-fermentive bacteria, 96STAPH for *Staphylococcus*, 96STREP for *Streptococcus* & *Enterococcus*, and 96FUNGUS for yeast (https://en.medicaldl.com/product/3.html). Each DL96 kit includes 5mL of ID broth, 10mL of AST broth and a 96-wells panel. 18 to 28 wells containing dehydrated substrates are for identification. Prepared bacterial suspension using ID broth is added into the ID wells and inoculated. Metabolism produces color changes that can be read (some changes may need additional reagents) after incubation, and identification is got after inputting biochemical reaction results into the DL software. The remaining 68 to 78 wells are for AST, containing different antimicrobial at 2–5 double-diluted concentrations. 50μL of bacteria in the ID broth is transferred and 200-fold diluted into the AST broth, and

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inoculated into the AST wells and cultured overnight. Bacterial growth is determined based on turbidity, and Minimum Inhibitory concentration (MIC) and susceptibility results is obtained using DL software. In this study, we assessed the performance of the 96E kit for *Enterobacteriales* using the broth microdilution (BMD) as a reference method.

Materials and Methods

Strains and Identification

A total of 270 *Enterobacterales* isolates were utilized, including strains isolated between June and August 2021 from Hainan general hospital, and some stored isolates (*Morganella, Salmonella* and Carbapenem-resistant *Enterobacterales*) as challenge strains. The specific distribution of the 270 *Enterobacterales* isolates was: 78 *Escherichia coli*, 79 *Klebsiella spp* (comprising 68 *Klebsiella pneumoniae*, four *Klebsiella oxytoca*, and seven *Klebsiella aerogenes*), 47 *Morganellaceae* (including 22 *Proteus mirabilis*, six *Proteus vulgaris/penneri*, 13 *Morganella morganii*, five *Providencia rettgeri*, and one *Providencia alcalifaciens*), 29 *Salmonella spp*, and 37 other *Enterobacteriaceae* (including 16 *Enterobacter cloacae* complex, 14 *Serratia marcescens*, six *Citrobacter spp*, and one *Leclercia adecarboxylata*). VITEK-MS (BioMérieux, Marcy l'Étoile, France) was used to identify bacteria other than *Salmonella* at species level.

Before the susceptibility test, all clinical isolates were subcultured on Columbia blood medium (BAP, Autobio Zhengzhou China) and confirmed pure culture. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 35218, and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains. Quality control for DL96E and BMD was performed weekly during the test.

BMD

According to Clinical and Laboratory Standards Institute (CLSI) M07 requirements,[3] BMD panels were prepared with 100 μL volume per well by the Institute of Antibiotics, Huashan Hospital, Affiliated with Fudan University. All antimicrobial agents were purchased from China National Institutes for Food and Drug Control (China national institutes for drug control) as National Standards. The antimicrobial agent concentrations were as follows: Trimethoprim-sulfamethoxazole (0.5/9.5–16/304 μg/mL), ampicillin/sulbactam (1/0.5–32/16 μg/mL), cefoperazone/sulbactam (1/0.5–32/16 μg/mL), piperacillin/tazobactam (2/4–64/4 μg/mL), ticarcillin/clavulanic acid (2/2–64/2 μg/mL), levofloxacin (0.12–4 μg/mL), ciprofloxacin (0.06–2 μg/mL), meropenem (0.5–8 μg/mL), imipenem (0.5–16 μg/mL), cefuroxime (1–32 μg/mL), cefazolin (1–32 μg/mL), ceftriaxone (1–32 μg/mL), cefepime (0.5–16 μg/mL), ceftazidime (1–32 μg/mL), cefoxitin (1–32 μg/mL), gentamicin (0.5–8 μg/mL), ampicillin (1–16 μg/mL), chloramphenicol (1–32 μg/mL), amikacin (1–32 μg/mL), and minocycline (0.25–8 μg/mL). The prepared panels were stored at −70°C and left at room temperature for 30 minutes before use. AST was operated under the standard procedures of CLSI M07[2] methods for aerobic bacteria.

DL96

DL96 susceptibility testing were performed following DL96E ifu.[4] Three to five pure colonies were selected to prepare a 0.5 McFarland bacterial suspension using the ID broth provided in the kit. Briefly, 50μL of the suspension was diluted 200-fold with 10mL of AST broth, then 100μL of AST broth was added to each AST well. After 16–20 hours of incubation at 35°C, DL96 Minimum inhibitory concentration (MIC) was read and double-checked manually. Susceptible (S), intermediate (I or susceptible dose-dependent SDD), and resistant (R) were determined using the DL software based on the breakpoints.

Data Processing and Analysis

Except for cefoperazone/sulbactam, all antimicrobials were analyzed uniformly with breakpoints in CLSI M100[5] and results were analyzed following CLSI M52[6] standard. The essential agreement (EA) was unidentified because the gradients of MIC tests for most antibiotics of DL 96 were less than five serial doubling dilutions. Categorical Agreement (CA), Very Major Errors (VME, false susceptible), Major Errors (ME, false resistant), and minor Errors (mE, errors about intermediate) were primarily analyzed.[6,7] Cefoperazone/sulbactam was evaluated and analyzed using breakpoints from the antibiotics ifu.[8] Intrinsic resistance was not statistically analyzed.

Error Check

All ME and VME should be reviewed. If the error still exists, record it as VME or ME. If the error was unrepeatable, the first result should be ignored and excluded from the error scope. Data with consistent BMD results would be considered.

Error Analysis of DL Missed Carbapenem Resistant Enterobacteriales (CRE)

All CRE strains were tested with mCIM[9] to determine the presence of carbapenemase. NG-test Carba 5 (Fosun Diagnostics Shanghai China) was used to classify carbapenemase. DL96 missed CRE error was analyzed based on these results.

Results

The accuracy of 20 antimicrobial agents was compared. CA ranged from 63.9% to 96.5%, with imipenem exhibiting the lowest CA at 63.9% and ampicillin the highest CA at 96.5% (Table 1). Additionally, the proportion of VME was high. Only ampicillin, levofloxacin, and cefazolin had a VME less than 3%, while the remaining 17 antimicrobial had VME greater than 3%, with imipenem having the highest VME at 52.8%.

The carbapenems results were subdivided further. A total of 56 VME isolates of imipenem-none-susceptible *Enterobacterales* were detected, including *Morganella* (39 isolates), *E. coli* (six isolates), *Klebsiella* (four isolates) and other *Enterobacterales* (seven isolates). There were 10 VME isolates of meropenem-none-susceptible *Enterobacterales*, including *E. coli* (two isolates), *Klebsiella* (one isolate), *Morganella* (one isolate), *Salmonella* (one isolate), and other *Enterobacterales* (five isolates) (Table 2).

103 isolates of CRE were evaluated, including *E. coli* (25 isolates), *Klebsiella spp*. (39 isolates), other *Enterobacterales* (23 isolates), *Salmonella spp*. (11 isolates), and meropenem-resistant *Morganella* (five isolates). DL 96 failed to detect 22 isolates, including *E. coli* (two isolates), *K. pneumoniae* (three isolates); *K. aerogenes* (one isolates), *Enterobacter cloacae* complex (one isolates), *Serratia marcescens* (seven isolates), *Salmonella spp* (five isolates), *Morganella morganii* (one isolate), *Proteus mirabilis* (one isolate), and *Proteus vulgaris*/*penneri* (one isolate).

Table 1 The Accuracy Rate of Drug Susceptibility of 270 *Enterobacterales* Isolates

Antimicrobial Agents	Number	S (S%)	R (R%)	CA	mE (mE%)	ME (ME%)	VME (VME%)
Amikacin	241	202 (83.8%)	31 (12.9%)	93.4%	8 (3.3%)	3 (1.5%)	5 (16.1%)
Gentamicin	239	152 (63.6%)	78 (32.6%)	86.6%	13 (5.4%)	5 (3.3%)	14 (17.9%)
Ampicillin	254	33 (13.0%)	217 (85.4%)	96.5%	4 (1.6%)	4 (12.1%)	1 (0.5%)
Ampicillin/Sulbactam	259	59 (22.8%)	165 (63.7%)	64.5%	67 (25.9%)	1 (1.7%)	24 (14.5%)
Cefoperazone/Sulbactam	269	173 (64.3%)	82 (30.5%)	86.6%	22 (8.2%)	0 (0.0%)	14 (17.1%)
Ticarcillin/Clavulanic acid	269	118 (43.9%)	100 (37.2%)	73.2%	62 (23.0%)	2 (1.7%)	8 (8.0%)
Piperacillin/Tazobactam	270	179 (66.3%)	75 (27.8%)	87.4%	15 (5.6%)	0 (0.0%)	19 (25.3%)
Cefazolin[a]	255	52 (20.4%)	188 (73.7%)	92.9%	14 (5.5%)	4 (7.7%)	0 (0.0%)
Cefuroxime	254	83 (32.7%)	158 (62.2%)	89.8%	12 (4.7%)	6 (7.2%)	8 (5.1%)
Ceftazidime	266	153 (57.5%)	99 (37.2%)	83.1%	21 (7.9%)	8 (5.2%)	16 (16.2%)
Ceftriaxone	269	126 (46.8%)	137 (50.9%)	90.0%	7 (2.6%)	5 (4.0%)	15 (10.9%)
Cefepime	270	136 (50.4%)	114 (42.2%)	77.8%	28 (10.4%)	1 (0.7%)	31 (27.2%)
Cefoxitin	260	111 (42.7%)	106 (40.8%)	74.6%	44 (16.9%)	4 (3.6%)	18 (17.0%)
Imipenem	269	134 (49.8%)	106 (39.4%)	63.9%	41 (15.2%)	0 (0.0%)	56 (52.8%)
Meropenem	269	203 (75.5%)	60 (22.3%)	92.9%	9 (3.3%)	0 (0.0%)	10 (16.7%)
Ciprofloxacin	241	76 (31.5%)	146 (60.6%)	81.7%	38 (15.8%)	1 (1.3%)	5 (3.4%)
Levofloxacin	241	86 (35.7%)	130 (53.9%)	81.3%	40 (16.6%)	2 (2.3%)	3 (2.3%)
Minocycline	268	145 (54.1%)	73 (27.2%)	68.7%	64 (23.9%)	3 (2.1%)	17 (23.3%)
Trimethoprim-sulfamethoxazole	213	109 (51.2%)	104 (48.8%)	92.0%	0 (0.0%)	11 (10.1%)	6 (5.8%)
Chloramphenicol	225	88 (39.1%)	106 (47.1%)	78.2%	38 (16.9%)	1 (1.1%)	10 (9.4%)

Note: [a]Group A breakpoints of CLSI are used uniformly for cefazolin.

Table 2 The Accuracy Rate of 270 *Enterobacterales* Isolates for Carbapenems

Antimicrobial Agents	Species	Number	S (S%)	R (R%)	CA	mE (mE%)	ME (ME%)	VME (VME%)
Imipenem	*Escherichia coli*	78	55 (70.5%)	15 (19.2%)	79.5%	10 (12.8%)	0 (0.0%)	6 (40.0%)
	Klebsiella	79	42 (53.2%)	32 (40.5%)	88.6%	5 (6.3%)	0 (0.0%)	4 (12.5%)
	Morganellaceae	47	0 (0.0%)	43 (91.5%)	2.2%	6 (12.8%)	0 (/)	39 (90.6%)
	Salmonella	29	22 (75.9%)	0 (0%)	75.9%	7 (24.1%)	0 (0.0%)	0 (/)
	Other *Enterobacteriaceae*	37	15 (40.5%)	17 (45.9%)	45.9%	13 (35.1%)	0 (0.0%)	7 (41.2%)
Meropenem	*Escherichia coli*	78	65 (83.3%)	11 (14.1%)	94.9%	2 (2.6%)	0 (0.0%)	2 (18.2%)
	Klebsiella	79	47 (59.5%)	30 (38.0%)	96.2%	2 (2.5%)	0 (0.0%)	1 (3.3%)
	Morganellaceae	47	43 (91.5%)	2 (4.3%)	93.6%	2 (4.3%)	0 (0.0%)	1 (50.0%)
	Salmonella	29	28 (96.6%)	1 (3.4%)	96.6%	0 (0.0%)	0 (0.0%)	1 (100.0%)
	Other *Enterobacteriaceae*	37	21 (56.8%)	16 (43.2%)	78.4%	3 (8.1%)	0 (0.0%)	5 (31.3%)

Table 3 Error Analysis of DL Missed CRE

No	Species	DL		BMD		mCIM*	Carbapenemase*
		IPM	MEM	IPM	MEM		
1	*Klebsiella pneumoniae*	≤ 1	≤ 1	≧ 64	≧ 64	POS	NDM
4	*Serratia marcescens*	≤ 1	≤ 1	8	16	NEG	NEG
6	*Klebsiella aerogenes*	≤ 1	≤ 1	4	0.12	NEG	NEG
7	*Proteus mirabilis*	≤ 1	≤ 1	16	2	NEG	NEG
8	*Serratia marcescens*	≤ 1	≤ 1	4	≧ 64	NEG	NEG
9	*Enterobacter cloacae* complex	≤ 1	≤ 1	4	1	NEG	NEG
15	*Escherichia coli*	≤ 1	≤ 1	4	≦ 0.006	NEG	NEG
18	*Serratia marcescens*	≤ 1	≤ 1	4	≦ 0.006	NEG	NEG
19	*Serratia marcescens*	≤ 1	≤ 1	2	≦ 0.006	POS	NDM
21	*Serratia marcescens*	≤ 1	≤ 1	≧64	≧ 64	POS	NDM
23	*Proteus vulgaris/penneri*	≤ 1	≤ 1	8	2	NEG	NEG
24	*Salmonella* sp.	≤ 1	≤ 1	2	≦ 0.006	POS	NDM
26	*Escherichia coli*	≤ 1	≤ 1	8	0.12	NEG	NEG
27	*Salmonella* sp.	≤ 1	≤ 1	2	≦ 0.006	NEG	NEG
28	*Salmonella* sp.	≤ 1	≤ 1	2	≦ 0.006	NEG	NEG
30	*Salmonella* sp.	≤ 1	≤ 1	2	≦ 0.006	NEG	NEG
31	*Salmonella* sp.	≤ 1	≤ 1	2	0.12	NEG	NEG
35	*Klebsiella pneumoniae*	≤ 1	≤ 1	1	2	NEG	NEG
36	*Serratia marcescens*	≤ 1	≤ 1	≧ 64	32	NEG	NEG
37	*Serratia marcescens*	≤ 1	≤ 1	≧ 64	≧ 64	POS	VIM
38	*Morganella morganii*	≤ 1	≤ 1	8	2	POS	VIM+KPC+OXA48
40	*Klebsiella pneumoniae*	≤ 1	≤ 1	4	2	NEG	NEG

Abbreviations: *POS, mCIM test positive; NEG, mCIM or NG5 Carba test negative.

Sixteen of these isolates were mCIM-negative, and six were mCIM-positive, among which four strains were NDM positive, one was VIM positive, and one was NDM+VIM+OXA48 positive (Table 3).

MIC distribution of the detected antimicrobial agents for *Enterobacterales* were shown in Figures 1–6. Figure 1 for Fluoroquinolones (A ciprofloxacin, B levofloxacin), Figure 2 for Carbapenems (A imipenem, B meropenem), Figure 3 for Cephems (A cefazolin, B cefuroxime, C ceftriaxone, D ceftazidime, E cefepime and F cefoxitin), Figure 4 for Penicillins and β-lactam combination agents (A ampicillin, B ampicillin/sulbactam, C cefoperazone/sulbactam, D ticarcillin/clavulanic acid and E piperacillin/tazobactam), Figure 5 for Aminoglycosides (A amikacin, B gentamicin) and Figure 6 for other antibiotic (A sulfamethoxazole, B minocycline, C chloramphenicol).

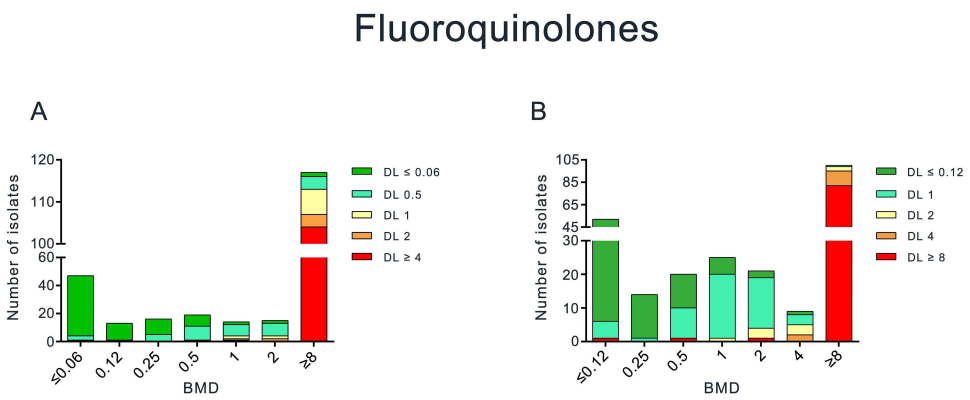

Figure 1 MIC distribution of Fluoroquinolones for *Enterobacterales* (except *Salmonella*) ((**A**) ciprofloxacin, (**B**) levofloxacin).

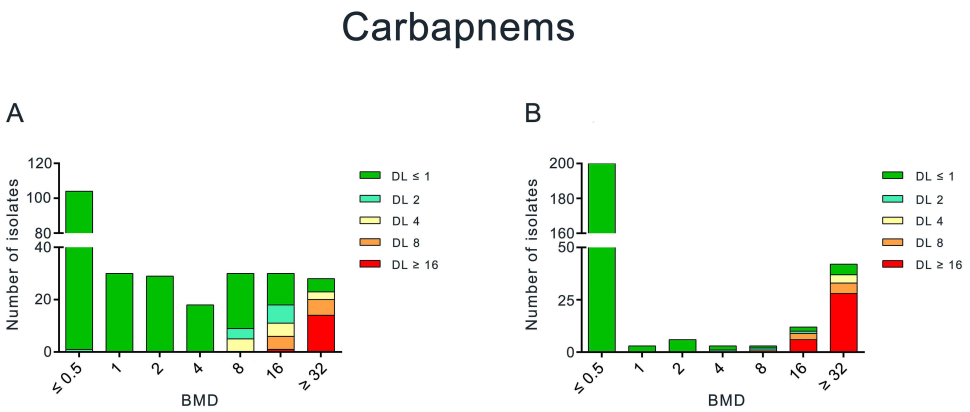

Figure 2 MIC distribution of Carbapenems for *Enterobacterales* ((**A**) imipenem, (**B**) meropenem).

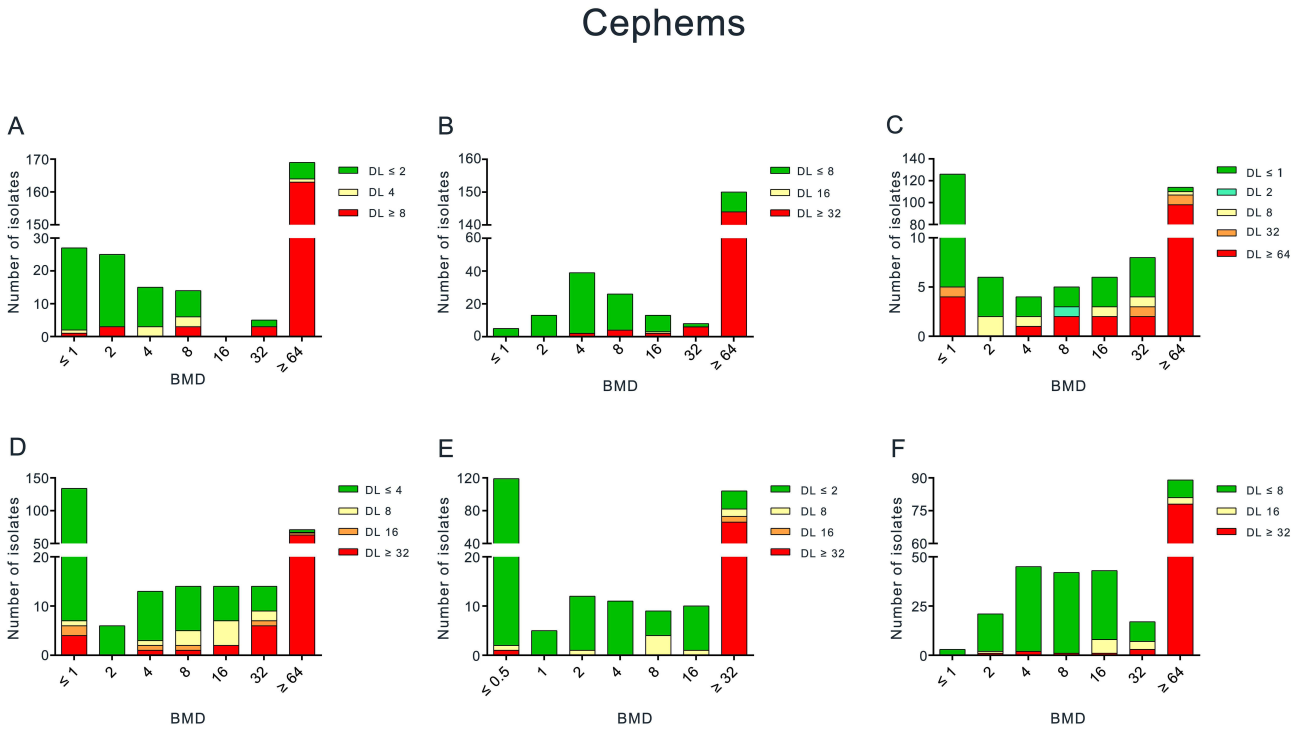

Figure 3 MIC distribution of Cephems for *Enterobacterales* ((**A**) cefazolin, (**B**) cefuroxime, (**C**) ceftriaxone, (**D**) ceftazidime, (**E**) cefepime and (**F**) cefoxitin).

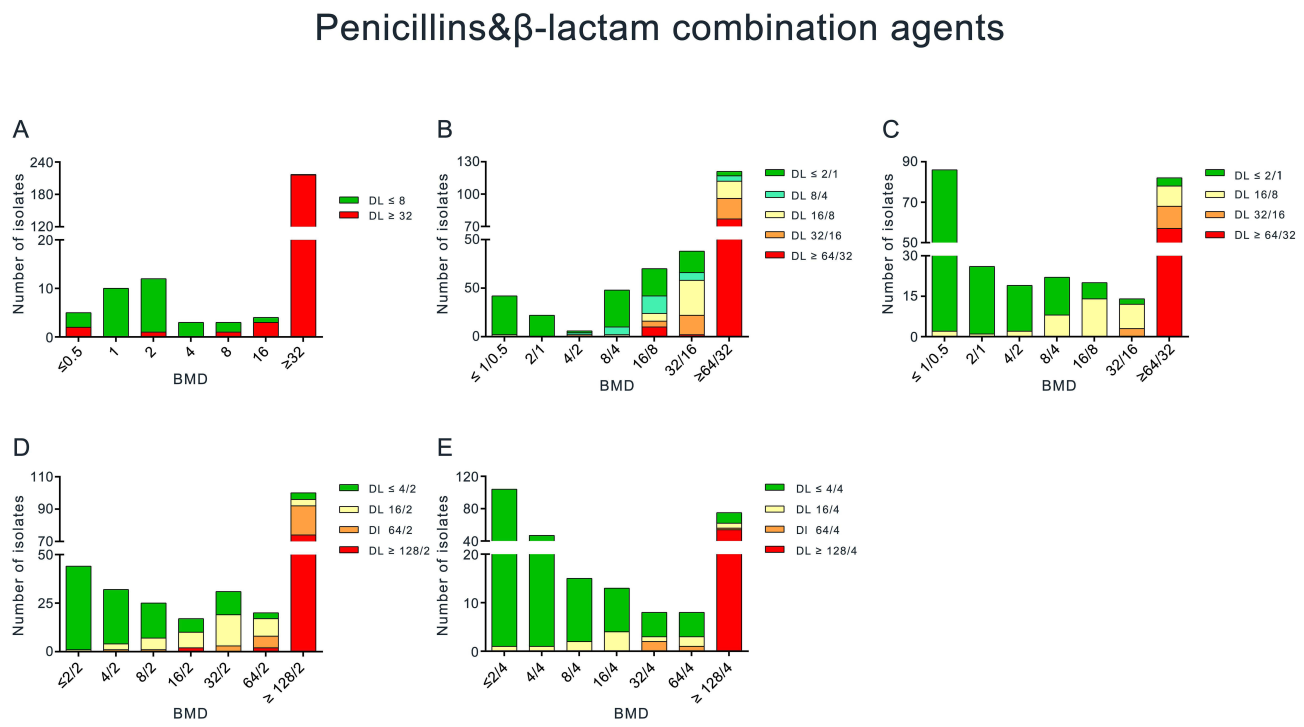

Figure 4 MIC distribution of Penicillins and β-lactam combination agents for *Enterobacterales* ((**A**) ampicillin, (**B**) ampicillin/sulbactam, (**C**) cefoperazone/sulbactam, (**D**) ticarcillin/clavulanic acid and (**E**) piperacillin/tazobactam).

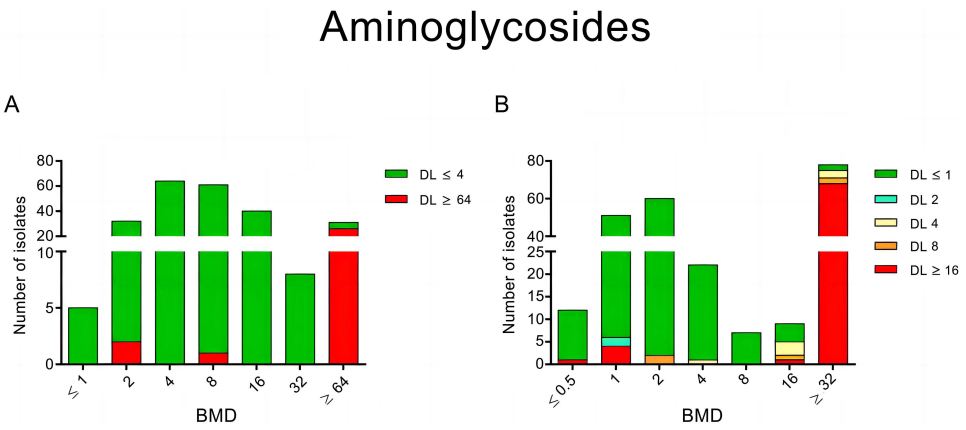

Figure 5 MIC distribution of Aminoglycosides for *Enterobacterales* ((**A**) amikacin, (**B**) gentamicin).

Discussion

In 2019, CLSI M100[5] adjusted the breakpoints of ciprofloxacin and levofloxacin for *Enterobacterales*. The new breakpoints of *Enterobacterales* to ciprofloxacin are: S ≤ 0.25 μg/mL, I = 0.5 μg/mL, and R ≥ 1 μg/mL, and to levofloxacin are: S ≤ 0.5 μg/mL, I = 1 μg/mL, and R ≥ 2 μg/mL. However, ciprofloxacin concentrations in DL 96E wells[3] were 0.06, 0.5, 1, and 2 μg/mL, and levofloxacin 0.12, 1, 2, and 4 μg/mL. Accordingly, the detected MICs do not cover the breakpoints and cannot distinguish between susceptible and intermediate isolates. This resulted in lower CA for ciprofloxacin and levofloxacin, and most errors are ME. Therefore, current DL 96E users should double-check isolates with MICs less than or equal to 0.5 μg/mL for ciprofloxacin and 1 μg/mL for levofloxacin (Figure 1). After CLSI changed the breakpoint in 2022, the same issue emerged with Piperacillin/tazobactam.

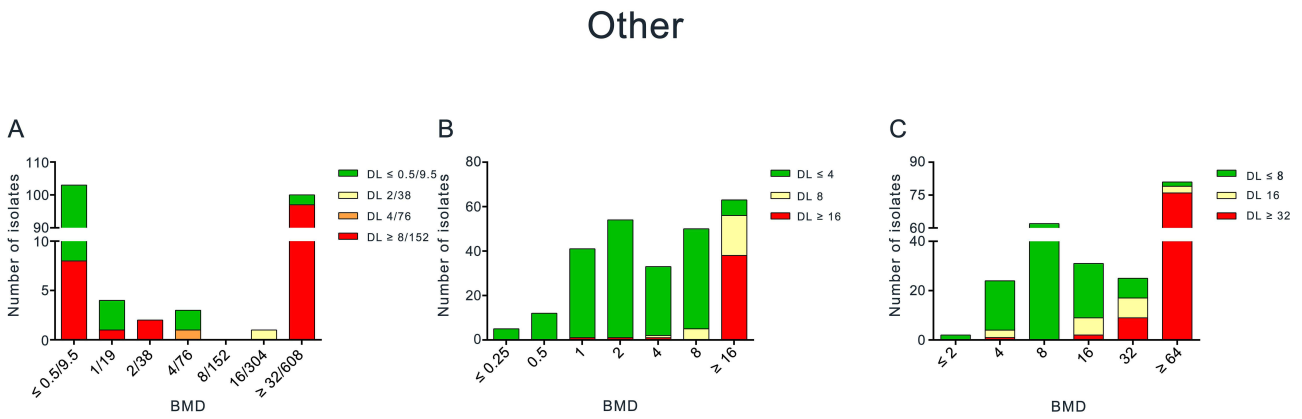

Figure 6 MIC distribution of other antibiotic for *Enterobacterales* ((**A**) sulfamethoxazole, (**B**) minocycline, (**C**) chloramphenicol).

CA of imipenem was 62.8%, and VME was up to 56.3%, Dr. Xu also found the same problem in a KPC-producing *Klebsiella pneumoniae*, whose CA was 59.1%.[2] The primary reason for this finding may be that the proportion of low level of imipenem resistant isolates was relatively high in this study. There were 79 low-level resistant isolates with MIC at 2–8 μg/mL, and ME (26 isolates) and VME (47 isolates) were both high (Figure 2A). As listed in Table 3, the MIC detected by DL 96E was two or three doubling dilutions lower than that of BMD, which is the cause of low CA and high VME (Table 3). In addition to the clinical isolates, several preserved isolates, especially *Morganellaceae*, including 22 *P. mirabilis* isolates, six *P. vulgaris*/*P. penneri* isolates, 13 *Morganella morganii* isolates, five *Providencia rettgeri* isolates, and one *Providencia alcalifaciens* strain, were selected due to the necessity of evaluating as many *Enterobacterales* bacteria as possible. CA of imipenem among *Morganellaceae* was only 2.2% while VME was 92.9%. This is related to the decrease of penicillin-binding protein (PBP), a special resistance mechanism of *Morganellaceae* to imipenem.[10] The MIC distribution of *Morganellaceae* for imipenem ranged from 1 to 4.[11] CLSI M100 has a comment in Appendix B[9] stating that susceptible isolates should be reported as susceptible.

Meropenem performed better than imipenem (90.3% for CA and 27.7% for VME). The primary reason is that *Morganellaceae* lacked a unique meropenem-resistance mechanism. In this evaluation, there were 17 low-level meropenem-resistant isolates with MIC at 2–8 μg/mL, less than 79 of imipenem (Figure 2B). Among the 17 isolates, five were ME, and 10 were VME, with an error rate of 88%. Tests for low-level resistant isolates should be addressed. Further studies must determine whether these errors are related to different resistance mechanisms or the carbapenemase enzymatic type. Since carbapenems are still one of the first-line antimicrobial for treating severe Gram-negative bacterial infections,[11] laboratories should consider other alternative methods to test carbapenem susceptibility of *Enterobacterales*.

Table 3 indicates that 72.7% (16/22) of the DL missed CREs isolates did not produce carbapenemases. Nine isolates were resistant to imipenem but sensitive to meropenem, one isolate was sensitive to imipenem but intermediate to meropenem, and the remaining six isolates were resistant to both imipenem and meropenem. The carbapenems' MIC of 12 isolates was 2–8 μg/mL. Low-level resistance may account for DL 96 failure to detect CRE; Other four isolates contained at least one carbapenem whose MIC were greater than or equal to 16 μg/mL, the reason for missed detection needs further investigation. The other six isolates DL 96 missed detected were Carbapenemase-producing *Enterobacteriaceae* (CPE). Three isolates' carbapenems MIC was between 2 and 8 μg/mL. Low-level resistance may also be the cause of DL 96 detection failure. *S. marcescens* (two isolates) and *K. pneumoniae* (one isolate) had imipenem and meropenem MIC ≥ 64 μg/mL, the reason for missed detection needs further investigation.

Although CRE is a most desirable multi-drug resistant organism (MDRO),[12] CPE is important in hospital-acquired infection. However, the missed detection rate of CRE for DL was 21.4% (22/103), including 6 CPE. Therefore, we recommend that all DL96 users use alternative methods to confirm carbapenem results.

The CA of other antimicrobials, including Gentamicin, Ampicillin/Sulbactam, Cefoperazone/Sulbactam, Ticarcillin/Clavulanic acid, Piperacillin/Tazobactam, Cefuroxime, Ceftazidime, Cefepime, Cefoxitin, Minocycline and

Chloramphenicol were lower than 90%. One possible reason is that the DL96 system has only 2–3 gradients for these antimicrobial, and some antimicrobial gradients exhibit discontinuous double dilution[4] (Figures 3–6). In addition, the concentrations of cefazolin in DL 96E wells[3] were 2 and 4 μg/mL, of cefuroxime were 8 and 16 μg/mL, which do not cover the Group U breakpoints of CLSI. Therefore, it is necessary to confirm these antimicrobial results by other methods.

Despite the poor performance of DL 96E AST, each batch of DL96E cards passed AST quality control (QC). It may be due to the narrow MIC detection range of some antimicrobial that cannot cover the MIC range of the QC strains.

Conclusion

Regarding the antimicrobial susceptibility test, DL 96E still needs improvement. The MIC ranges for ciprofloxacin, levofloxacin, and piperacillin/tazobactam must be adjusted according to CLSI design. The MIC gradients for cefazolin and cefuroxime must be increased to cover the Group U breakpoints of CLSI. Antimicrobial agent formulations for imipenem should be redesigned to solve the problem of overall deviation to reduce the missed detection of low-level resistant isolates. The MIC gradient should be increased to cover the scope of quality control for other antimicrobials, such as minocycline, chloramphenicol, and cefoperazone/sulbactam, which will increase the relative accuracy and readily identify control for QC assessment.

Data Sharing Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Ethics Approval and Informed Consent

The institutional review boards at the Hainan General Hospital approved the study protocol. The research objects are bacteria, not people or animals, so no informed consent is required.

Consent for Publication

The details of results and images can be published.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interest in this work.

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