Real-world Performance of Susceptibility Testing for Ceftolozane/Tazobactam Against Non-Carbapenemase-Producing Carbapenem-Resistant *Pseudomonas aeruginosa*

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Abstract.

Ceftolozane/ tazbactam (C/T) is a potent anti-pseudomonal agent that has clinical utility against infections caused by non-carbapenemase producing carbapenem-resistant *P. aeruginosa* (non-CP-CR-PA). Accurate, precise and reliable antimicrobial susceptibility testing (AST) is crucial to guide clinical decisions. However, studies assessing the performance of different AST methods against non-CP-CR-PA- (the main clinical niche for C/T), are lacking. Here, we evaluated performance of gradient strips (Etest and MIC test strip (MTS), and disk diffusion (DD) using CLSI breakpoints. Additionally, we assessed the performance of DD using EUCAST breakpoints. For all susceptibility tests, we used a collection of 97 non-CP-CR-PA clinical isolates recovered from 11 Chilean hospitals. Both gradient strips and DD had acceptable performance when using CLSI breakpoints, yielding a categorical agreement (CA) of >90% and 92%, respectively. In contrast, DD using EUCAST breakpoints performed sub-optimally (CA 81%). MTS yielded a higher essential agreement (EA, >90%) than Etest (84%). Importantly, the performance of all methods varied significantly when the isolates were stratified by their degree of susceptibility to other anti-pseudomonal β-lactams. All methods had 100% CA when testing isolates that were pan-susceptible to all β-lactams (Pan-β-S). However, the CA markedly decreased when testing isolates resistant to all β-lactams (Pan-β-R). Indeed, the CA was 81% for Etest (6 errors), 78% for MTS (7 errors) and 78% and 56% for DD when using CLSI (7 errors) or EUCAST breakpoints (14 errors), respectively. Our results suggest that all manual AST methods have strikingly decreased performance in the context of Pan-β-R *P. aeruginosa* with potentially major clinical implications.

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Introduction

Ceftolozane-tazobactam (C/T) consists of the combination of ceftolozane, a novel oxyimino-cephalosporin, with tazobactam, an established beta-lactamase inhibitor. C/T is approved for the treatment of complicated intra-abdominal and urinary tract infections, and hospital-associated pneumonia (1–3). Ceftolozane exhibits high affinity for multiple essential *P. aeruginosa* penicillin-binding proteins and can remain active in the presence of mutations encoding upregulated efflux pumps, porin loss and constitutive production of the AmpC beta-lactamase, all of which are frequently observed in multidrug resistant *P. aeruginosa* (3, 4). In contrast, C/T is not active against carbapenemase-producing organisms (5). Several large-scale studies have shown C/T is highly active *in vitro* against the majority of clinical isolates of *P. aeruginosa,* and is particularly valuable against non-carbapenemase-producing, carbapenem-resistant *P. aeruginos*a (non-CP-CR-PA) (3, 6–8). However, development of resistance to C/T during therapy and *de novo* has been reported. Rates of resistance to C/T have been increasing over time (8–10). More importantly, minimum inhibitory concentrations (MICs) close to the clinical breakpoint (4-8 µg/mL) have been associated with a higher risk of therapeutic failure (11–14). Thus, the availability of accurate, precise and reproducible susceptibility testing methods for evaluation of C/T activity for clinical laboratories to effectively guide clinical decision making is of paramount importance for patient care.

Previous studies assessing manual antimicrobial susceptibility testing (AST) methods (i.e. Etest, MIC Test Strips [MTS] and disk diffusion [DD]) for C/T have shown variable performance relative to the gold standard, broth microdilution (BMD) (15–18). Of note, only one of these studies compared all three methods in parallel (16). In addition, performance of DD has

not been evaluated to date using EUCAST breakpoints. Most importantly, all existing studies utilized *P. aeruginosa* isolates with diverse phenotypic and genotypic profiles, with few of them focusing on non-CP-CR-PA, which are the unique niche of organisms that C/T has clinical therapeutic value against.

Here, we evaluated the performance of widely used manual C/T susceptibility testing methods that included gradient strips (Etest [bioMerieux]; MTS [Liofilchem]) and DD using CLSI and EUCAST breakpoints, against a collection of 97 non-CP-CR-PA clinical isolates from 11 Chilean hospitals. In addition, we assessed the performance of these methods by stratifying the non-CP-CR-PA isolates according to their degree of susceptibility to other anti-pseudomonal β-lactams.

Methods

Isolate collection and carbapenemase screening. Clinical isolates of carbapenem-resistant *P. aeruginosa* (CR-PAE) were prospectively collected at 11 tertiary-care Chilean hospitals in two time-periods: August to October, 2017 and December 2018 to November 2019. Only the first isolate was obtained per patient; surveillance cultures were not included. All isolates were sent to a central laboratory for further testing. Species identification was confirmed using MALDI-TOF (VITEK® MS bioMérieux, Durham, NC). Isolates were catalogued as carbapenem-resistant if they exhibited resistance to either imipenem (IMI), meropenem (MEM), or both. Since the focus of our work was on non-CP-CR-PA, we performed a two-step approach to rule out carbapenemase-producing organisms. *First*, isolates were phenotypically screened for carbapenemase activity with BlueCarba as previously reported (19), and all positive strains were excluded. *Second*, BlueCarba-negative isolates were further screened for the presence of bla_{KPC},

98 *bla*$_{VIM}$, *bla*$_{NDM-1}$, *bla*$_{IMP}$ and *bla*$_{OXA-48}$ using a multiplex qPCR test. A list of primers and details of
99 the qPCR assay are provided in Table S1.
100
101 *Antimicrobial susceptibility testing*. Susceptibility testing for anti-pseudomonal antibiotics other
102 than C/T was performed using the DD method following CLSI recommendations (20) and
103 included: IMI, MEM, aztreonam (ATM), ceftazidime (CAZ), cefepime (FEP), piperacillin-
104 tazobactam (TZP), ciprofloxacin (CIP), amikacin (AMK) and gentamicin (GEN). C/T
105 susceptibility was assessed by BMD as per CLSI guidance (20). In-house panels were prepared
106 using cation-adjusted Mueller-Hinton broth (Becton Dickinson [BD]) spanning a ceftolozane
107 doubling dilution range of 0.125 to 64 µg/mL. A fixed tazobactam concentration of 4 µg/mL was
108 used throughout the dilution range. MIC values were read after incubation for 16-20h at 35°C.
109 C/T susceptibility with Etest and MTS was performed on MH agar (BD), as per manufacturers'
110 instructions with incubation at 35°C for 16-18h. Finally, C/T DD (Hardy Diagnostics) was
111 carried out with 30/10 µg discs following CLSI recommendations. All susceptibility techniques
112 were assessed in parallel using the same inoculum. Experiments were performed in duplicate; in
113 case of discordant results a third repetition was done and the highest repeated value was chosen
114 for the analysis. Quality control testing was performed for each experiment with *P. aeruginosa*
115 ATCC 27853 (C/T MIC range 0.25/4 – 1/4 µg/mL). Susceptibilities by BMD, Etest and MTS
116 were analyzed using the 2020 CLSI breakpoints (≤4/4, 8/4 and ≥16/4 µg/mL for C/T susceptible,
117 intermediate, and resistant, respectively). Performance of DD against BMD was assessed using
118 both EUCAST (≥24 mm susceptible and <24 mm resistant) and CLSI breakpoints (≥21 mm
119 susceptible, 17-20 mm intermediate and ≤16 mm resistant) (21).

120 *Data analysis*. C/T susceptibility results with each technique were compared and categorized
121 using BMD as the reference method. Categorical agreement (CA), essential agreement (EA),
122 major errors (ME), very major errors (VME) and minor errors (mE) were calculated. EA was
123 defined as agreement within ±1 dilution of the method under evaluation with BMD. E-test and
124 MTS MICs were rounded up to the next concentration of the standard doubling dilution scale
125 when necessary (e.g. 1.5 µg/mL was considered as 2 µg/mL). CA was defined as agreement of
126 interpretative results between the method under evaluation and BMD using CLSI or EUCAST
127 interpretive criteria, as appropriate. Discrepancies between the method under evaluation and
128 BMD were categorized as follows: VME, the method under evaluation indicated susceptible
129 while BMD indicated resistant; ME, the method under evaluation indicated resistant and BMD
130 indicated susceptible and mE, a discrepancy between the test and reference methods involved an
131 intermediate result (22). Finally, to further dissect the performance of susceptibility techniques in
132 the context of susceptibility to anti-pseudomonal β-lactams other than C/T (*i.e.* CAZ, FEP, TZP
133 and ATM), we classified our collection of non-CP-CR-PA into the following mutually exclusive
134 groups: ***i)*** isolates that remained susceptible to all other tested β-lactams in spite of exhibiting a
135 CR phenotype (Pan-β-S), ***ii)*** CR strains exhibiting resistance to some, but not all β-lactams (β-
136 R/S), and ***iii)*** CR strains exhibiting resistance to all tested β-lactams (*i.e*., pan-β-lactam-resistant
137 [Pan-β-R]).

138 **Results**

139 *Characteristics of the strain collection of clinical P. aeruginosa isolates*.
140 Out of 159 clinical isolates of CR-PA collected in the study period, we excluded 62 isolates
141 based on phenotypic or molecular detection of a carbapenemase; and included a total of
142 confirmed 97 non-CP-CR-PA in the study. Clinical sources included tissue/bone (n=30; 31%),

blood (n=18; 19%), skin (n=16; 16%), other sterile fluids (n=13; 13%), respiratory tract (n=11; 11%) and urine (n=9; 9%). The overall susceptibility profile of the non-CP-CR-PA isolates is shown in **Fig. 1**. Of the 97 isolates, 78 (80%) were non-susceptible to both IMI and MEM, whereas the remaining 19 isolates (20%) exhibited susceptibility to one of the carbapenems (15 susceptible to MEM only and 4 susceptible to IMI only) **(Fig. 2)**. To evaluate C/T activity against non-CP-CR-PA isolates and better assess the performance of susceptibility testing methodologies based on susceptibility patterns, isolates were categorized into the following 3 groups based on their susceptibility to β-lactams: ***i)*** Pan-β-R (n=26; 27%), isolates resistant to all β-lactams, ***ii)*** β-R/S (n=35, 36%), isolates susceptible to at least one anti-pseudomonal β-lactam and, ***iii)*** Pan-β-S (n=36, 37%), isolates susceptible to all tested β-lactams except for at least one of the carbapenems (**Table 1 and Fig. 2B)**.

C/T activity against non-CP-CR-PA decreases in pan β-lactam-resistant strains.

Overall, out of the 97 non-CP-CR-PA isolates, BMD categorized 87 (90%) as C/T susceptible, 6 (6%) as resistant and 4 (4%) as intermediate (**Table 1**). Isolates resistant to both carbapenems (n=78) displayed decreased susceptibility to C/T as well relative to the remaining 19 strains that were susceptible to one of the carbapenems (C/T $MIC_{50/90}$ 2/8 and 1/4 μg/mL, respectively) (**Fig. 2A**). As resistance to other anti-pseudomonal β-lactams increased, C/T $MIC_{50/90}$ values also exhibited an increasing trend, where Pan-β-S isolates had the lowest $MIC_{50/90}$ (1/2 μg/mL) followed by β-R/S (2/4 μg/mL) and Pan-β-R isolates (4/64 μg/mL) (**Table 1, Fig. 2B**). Similarly, isolates exhibiting a Pan-β-R phenotype had a higher frequency of C/T non-susceptibility (31%, n=8) as compared to their β-R/S or Pan-β-S counterparts, of which 97% remained C/T susceptible (**Table 1**).

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167 *Performance of Etest and MTS varied widely across different phenotypes of non-CP-CR-PA*.

168 The performance evaluation of C/T gradient strips using CLSI breakpoints yielded an
169 overall CA and EA of 93% and 84% for Etest, and 91% and 92% for MTS (**Table 2**). All isolates
170 out of EA (n=25 with Etest and n=7 with MTS) had MICs ≥ 2 dilutions lower than BMD,
171 suggesting both gradient strips overcall C/T susceptibility in non-CP-CR-PA (**Fig. 3 and Fig. 4**).

172 Notably, the performance of gradient strips varied widely when stratifying the non-CP-
173 CR-PA collection according to their susceptibility to other anti-pseudomonal β-lactams. While
174 both Etest and MTS used for Pan-β-S isolates yielded a CA of 100% and an EA of 86% and
175 92%, respectively, their performance decreased sharply in the Pan-β-R population, with CA of
176 77% for Etest and 73% for MTS, and an EA of 69% and 85% for Etest and MTS, respectively
177 (**Table 2**). Worrisomely, this decreased performance was primarily due to a failure of gradient
178 strips to detect isolates that were C/T non-susceptible by BMD in the Pan-β-R population (**Fig.**
179 **4**). Indeed, while 31% of the Pan-β-R isolates were non-susceptible by BMD, only 15% were
180 categorized as such by MTS and 8% by Etest (**Fig. 4**). Disagreement in C/T susceptibility results
181 between gradient strips and BMD was more pronounced at higher BMD MIC values. Etest and
182 MTS yielded a 100% EA and CA for strains with C/T MIC values ≤1 µg/mL, but their
183 performance progressively decreased, with both tests demonstrating CA in only 30% of isolates
184 with C/T BMD MIC ≥8 µg/mL **(Table 3, Fig. 3**).

185 Overall, we observed 3 VME with Etest and 2 with MTS; no ME were found. A total of 4
186 and 7 mE were observed with Etest and MTS, respectively (**Table 4**). Importantly, most errors
187 with both strips were obtained within the Pan-β-R group, and none of them were observed in
188 isolates with a Pan-β-S phenotype (**Table 4**).

189

190 *Disk diffusion performance across CLSI and EUCAST breakpoints*.

191 The overall performance of DD yielded a CA of 92% with the CLSI breakpoints and 81%
192 when applying the EUCAST recommendations (**Table 2)**. DD under-called non-susceptibility
193 with CLSI criteria, categorizing 94% of isolates as C/T susceptible, as compared to 90%
194 susceptibility with BMD (**Fig. 5**). In contrast, a total of 75% of isolates were deemed C/T
195 susceptible by DD using the EUCAST breakpoints (**Fig. 5**).

196 As observed with gradient strip tests, performance of DD varied widely when stratifying
197 the non-CP-CR-PA collection as per susceptibility to other anti-pseudomonal β-lactams. Indeed,
198 while the CA of DD using the interpretations of both agencies was 100% for Pan-β-S isolates, it
199 sharply decreased in the Pan-β-R population to only 73% and 50% with CLSI and EUCAST,
200 respectively (**Table 2**). Among Pan-β-R isolates, the CLSI criteria for DD categorized 84% as
201 C/T susceptible, as compared to only 42% obtained with the EUCAST breakpoints (**Fig. 5**).

202 Overall, DD yielded 1 VME, no ME and 7 mE with CLSI, and 3 VME and 17 ME when
203 using EUCAST (**Table 4)**. Similar to gradient strips, most errors concentrated in the Pan-β-R
204 population and none of them were observed in Pan-β-S isolates (**Table 4)**. All ME observed
205 with EUCAST corresponded to isolates with BMD MICs between 1 - 4 µg/mL (**Fig. 6**).

206

207 **Discussion**

208 C/T has become an important addition to the therapeutic arsenal available to manage non-
209 CP-CR-PA infections (2). However, with increasing use of C/T in clinical settings, several
210 studies reported clinical failures associated with C/T non-susceptible strains (13, 14, 23).
211 Therefore, the availability of accurate, easily-accessible and reliable susceptibility techniques is

paramount to inform therapeutic decisions. Our simultaneous evaluation of available manual AST techniques for the assessment of C/T activity against non-CP-CR-PA suggests the performance of all methods varies according to the degree of β-lactam resistance, with Pan-β-R isolates consistently exhibiting the most variability between testing methods. In contrast, all three methods exhibited an excellent agreement when used to evaluate Pan-β-S strains.

As previously reported, the activity of C/T decreased in the subgroup of Pan-β-R isolates, compared to those remaining susceptible to at least one anti-pseudomonal β-lactam (6, 15, 18). Indeed, 8 out of the 10 isolates that resulted as C/T non-susceptible by BMD belonged to the Pan-β-R population (**Table 1**). Similarly, BMD C/T MICs shifted to yield higher MIC_{50}/MIC_{90} values for strains exhibiting resistance to both carbapenems as opposed to those remaining susceptible to one of them.

Our findings showed that the overall CA for Etest and MTS was >90%, which largely aligns with previous reports that evidenced a CA ≥ 87% for both techniques (6, 15, 18). Similarly, our assessment of DD using CLSI breakpoints revealed a CA of 92%. However, DD performance with EUCAST recommendations yielded a CA of only 81%, with most discordant isolates corresponding to strains catalogued as C/T susceptible by BMD and resistant by DD (**Fig. 5**). While our observed overall EA of 84% for Etest was similar to the 79% reported by Shields et al., these values are considerably lower than those observed in two other previous reports (96% and 98%) (15, 16). In contrast, our results yielded an overall EA for MTS >90%, which is higher than previously reported (16, 17). Importantly, all isolates falling out of EA with both gradient strip tests exhibited MICs ≥ 2 dilutions lower than BMD.

235 A key difference between our current study and previous reports is that our strain set
236 consisted only of non-CP-CR-PA. Humphries' study included strains resistant to 1 ≥ anti-
237 pseudomonal β-lactam and Schaumburg et al. analyzed organisms exhibiting a multidrug-
238 resistant phenotype, regardless of β-lactam resistance or carbapenemase production (16, 17).
239 Thus, performance variation among studies could be partially explained by differences in the
240 strain collections.
241
242 Our most important finding was the striking performance variation observed for all AST
243 methods when stratifying our non-CP-CR-PA collection by resistance to other anti-pseudomonal
244 β-lactams. In particular, as shown in Table 3 and Table 5, our results showed a drastic decrease
245 in performance in the subgroup of Pan-β-R strains as compared to those exhibiting a Pan-β-S
246 phenotype across all three methodologies. Similarly, performance of both gradient strips also
247 decreased in the subgroup of isolates exhibiting higher C/T MIC values by BMD, with a CA for
248 both Etest and MTS of 100% for strains with MICs ≤ 1 µg/mL, and a drastic decrease to 30%
249 CA for strains with MICs ≥ 8 µg/mL. Our findings are particularly troublesome since they
250 demonstrate that none of the manual methods currently available for clinical use show acceptable
251 and reliable performance to evaluate C/T susceptibility in the subgroup of Pan-β-R isolates,
252 where this drug is particularly useful and needed for the treatment of severe infections. Studies
253 have also shown that the most common mechanism of C/T nonsusceptibility is an accumulation
254 of multiple single nucleotide polymorphisms (SNPs) in PDC, the AmpC cephalosporinase,
255 leading to overproduction and structural modification of the enzyme in addition to the
256 acquisition of extended spectrum β-lactams (24, 25). This mechanism also yields cross resistance
257 to ceftazidime/ avibactam, all cephalosporins and penicillins which is expected given that C/T is

used to treat MDR infections (14, 25). This supports the notion that *P. aeruginosa* is a particularly challenging Gram-negative pathogen because it can repurpose pre-existing resistance mechanisms to adapt and survive in the presence of novel agents. Our data suggest that it is important to evaluate performance of AST methods by using sets of isolates that reflect the clinical situations in which the antimicrobial under investigation is likely to be used. Here, assessment of AST methods stratifying isolates based on relevant susceptibility profiles, in this case to anti-pseudomonal β-lactams, identified potential pitfalls in current manual testing techniques and suggests simplified C/T BMD testing may be preferable to inform clinical decision making.

There are several limitations to this study. First, we did not perform molecular epidemiology techniques to assess for genetic relatedness, therefore, we do not have information regarding the clonality of our strain set. In addition, although this is a multicenter study, all isolates belonged to Chilean hospitals and larger studies with geographically diverse strain sets are still essential. Finally, we only studied the presence of the most frequently encountered carbapenemases (*i.e.* bla_{KPC}, bla_{VIM}, bla_{NDM-1}, bla_{IMP} and bla_{OXA-48}). Although we cannot rule out the possibility that some isolates could harbor other carbapenemase genes, our two-step approach combining phenotypic and molecular techniques largely decreases this possibility.

In summary, while we observed a good overall agreement between AST methods and BMD for susceptible isolates, we found significant discrepancies between all methods evaluated when isolates were resistant to all other anti-pseudomonal β-lactams. Unfortunately, the latter are likely to be those for which clinicians are seeking accurate C/T susceptibility results to guide

treatment decisions. Thus, there is still a significant need for rapid, cost-effective testing strategies for non-CP-CR-PA that can deliver reliable results to the bedside.

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Table 1. Distribution of 97 non-CP-CR-PA isolates based on ceftolozane/ tazobactam (C/T) susceptibility determined by broth microdilution with CLSI M100 breakpoints.

Isolates parsed into the following categories: i) susceptible to all β-lactams (Pan-β-S) except imipenem or meropenem, ii) susceptible to at-least one β-lactam and nonsusceptible to others (β-R/S), iii) nonsusceptible to all β-lactams (Pan-β-R). *n*= number of isolates

Resistance Phenotype	Total isolates (%)	C/T MIC by BMD (µg/mL)			Distribution *(n)* of isolates by C/T susceptibility		
		MIC_{50}	MIC_{90}	Range	Susceptible	Intermediate	Resistant
Non-CP-CR-PA	97 (100)	1	4	0.5 - ≥ 64	88% (85)	6% (6)	6% (6)
Pan-β-S	36 (37)	1	2	0.5 - ≥ 64	97% (35)	-	3% (1)
β-R/S	35 (36)	2	4	0.5 - ≥ 64	97% (34)	-	3% (1)
Pan-β-R	26 (27)	4	64	1 - ≥ 64	69% (18)	15% (4)	15% (4)

Table 2. Categorical (CA) and Essential Agreement (EA) of gradient strips- *Etest, MTS,* and *disk diffusion (DD)* compared to reference broth microdilution with CLSI M100 breakpoints and EUCAST breakpoints for DD. Isolates parsed into the following categories: i) susceptible to all β-lactams (Pan-β-S) except imipenem or meropenem, ii) susceptible to at-least one β-lactam and non-susceptible to others (β-R/S), iii) non-susceptible to all β-lactams (Pan-β-R). *N*= number of isolates

Resistance Phenotype	N isolates	CA % (*n*)				EA % (*n*)	
		Etest	*MTS*	*DD CLSI*	*DD EUCAST*	*Etest*	*MTS*
Non-CP-CR-PA	97	93 *(90)*	91 *(88)*	92 *(89)*	81 *(79)*	84 *(81)*	92 *(89)*
Pan-β-S	36	100	100	100	100	86 *(31)*	92 *(33)*
β-R/S	35	97 *(34)*	94 *(33)*	97 *(34)*	83 *(29)*	91 *(32)*	97 *(34)*
Panβ-R	26	77 *(20)*	73 *(19)*	73 *(19)*	50 *(13)*	69 *(18)*	85 *(22)*

Table 3. Categorical (CA) and Essential Agreement (EA) for Etest and MTS relative to reference broth microdilution (BMD) with CLSI M100 breakpoints. Non-CP-CR-PA isolates parsed by range of C/T MICs determined by BMD. *N*= number of isolates

C/T MIC range by BMD (ug/ml)	N isolates	CA % (*n*)		EA % (*n*)	
		Etest	*MTS*	*Etest*	*MTS*
≤ 1	41	100	100	100	100
2 - 4	46	100	96 *(39)*	80 *(37)*	94 *(38)*
≥ 8	10	30 *(3)*	30 *(3)*	30 *(3)*	60 *(6)*

Table 4. Breakdown of error rates and discrepancies from *Etest, MTS,* and *disk diffusion* (DD). Isolates parsed into the following categories: i) susceptible to all β-lactams (Pan-β-S) except imipenem or meropenem, ii) susceptible to at-least one β-lactam and nonsusceptible to others (β-R/S), iii) nonsusceptible to all β-lactams (Pan-β-R). *N*= number of isolates

Resistance Phenotype	N isolates	Very Major Errors (%)				Major Errors (%)				Minor Errors (%)		
		Etest	*MTS*	*DD CLSI*	*DD EUCAST*	*Etest*	*MTS*	*DD CLSI*	*DD EUCAST*	*Etest*	*MTS*	*DD CLSI*
All Non-CP-CR-PA	97	3/5 *(60)*	2/5 *(40)*	1/5 *(20)*	3/9 *(33)*	0	0	0	17/69 *(25)*	4 *(4)*	7 *(7)*	7 *(7)*
Pan-β-S	36	0	0	0	0	0	0	0	0	0	0	0
β-R/S	35	1 *(100)*	1 *(100)*	0	0	0	0	0	6/34 *(18)*	0	1 *(3)*	1 *(3)*
Panβ-R	26	2/3 *(67)*	1/3 *(33)*	1/3 *(33)*	3/7 *(43)*	0	0	0	9/16 *(56)*	4 *(15)*	6 *(23)*	6 *(23)*

Figure legends

Figure 1. Overall distribution 97 non-CP-CR-PA isolates categorized based on susceptibility with CLSI M100 breakpoints. Amikacin (AK), gentamicin (CN), aztreonam (ATM), ceftazidime (CAZ), cefepime (CEF), ciprofloxacin (CIP), imipenem (IMI), meropenem (MEM) and piperacillin/tazobactam (TZP); susceptible (S), intermediate (I), resistant (R).

Figure 2. Distribution of ceftolozane/ tazobactam MIC values of 97 non-CP-CR-PA isolates determined by broth microdilution uing CLSI M100 breakpoints.

A) Isolates parsed into 2 categories- **i)** susceptible to imipenem (IMI) or meropenem (MEM) and **ii)** non-susceptible to both carbapenems (intermediate or resistant). **B)** Isolates parsed into three categories: **i)** susceptible to all β-lactams (Pan-β-S) except imipenem or meropenem**, ii)** susceptible to at-least one β-lactam and non-susceptible to others (β-R/S), **iii)** non-susceptible to all β-lactams (Pan-β-R)

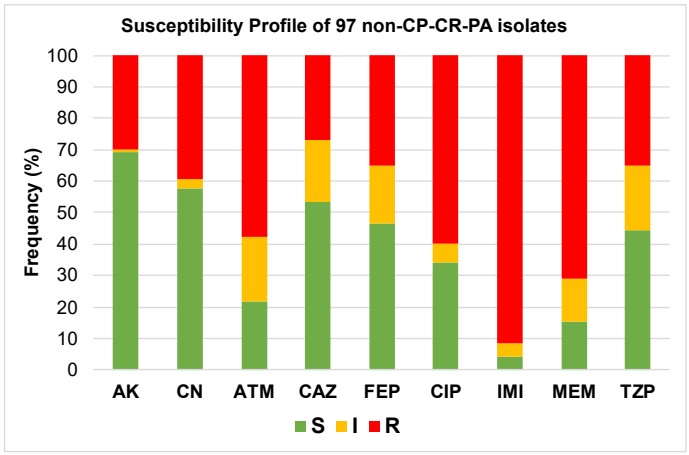
Figure 3. Correlation of MIC values (µg/mL) generated by reference broth microdilution (BMD) with those determined by A) Etest or B) MTS. Isolates in essential agreement indicated with shading. Isolates in categorical agreement are in black, very major errors in red, major errors in green and minor errors in blue.

Figure 4. Comparison of the frequency of C/T susceptibility in non-CP-CR-PA isolates by BMD, Etest and MTS based on CLSI M100 breakpoints. Isolates are divided into sub-categories based on their β-lactam susceptibility profile.

Figure 5. Correlation between MIC (µg/mL) values obtained with broth microdilution (BMD) and disk diffusion zone diameters as per CLSI (A) and EUCAST (B) breakpoints. A zone diameter of $\geq$ 21mm is considered susceptible (light blue), between 20 and 17mm intermediate (yellow) and $\leq$ 16mm resistant (pink).

Figure 6. Comparison of the the frequency of C/T susceptibility in non-CP-CR-PA isolates by BMD and DD based on CLSI M100 or EUCAST breakpoints. Isolates are divided into sub-categories based on their β-lactam susceptibility profile.

485 **Figure 1.**



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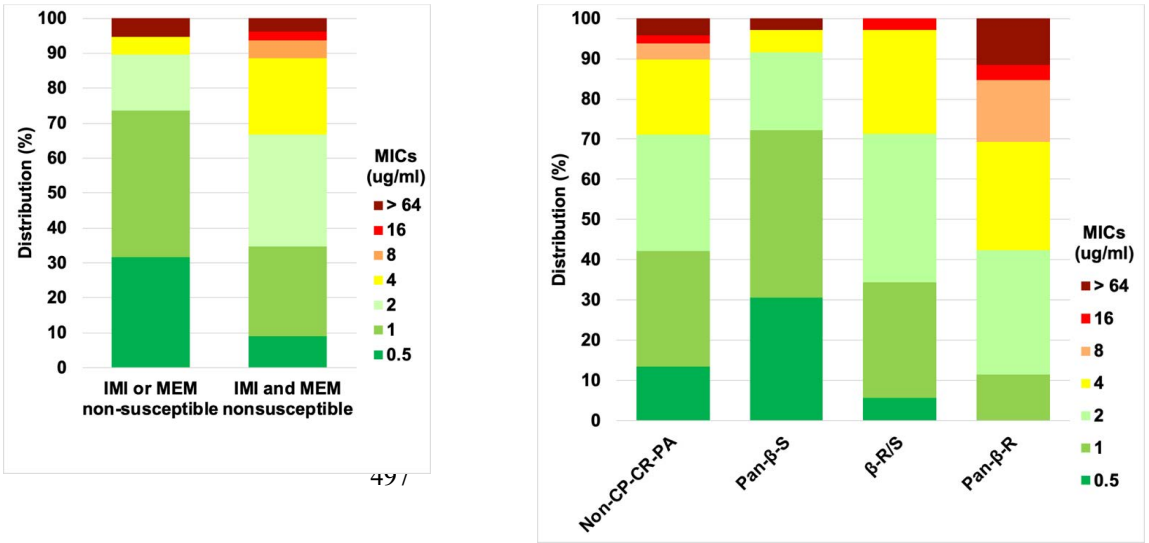
488 **Figure 2.**

489 **A.** **B.**



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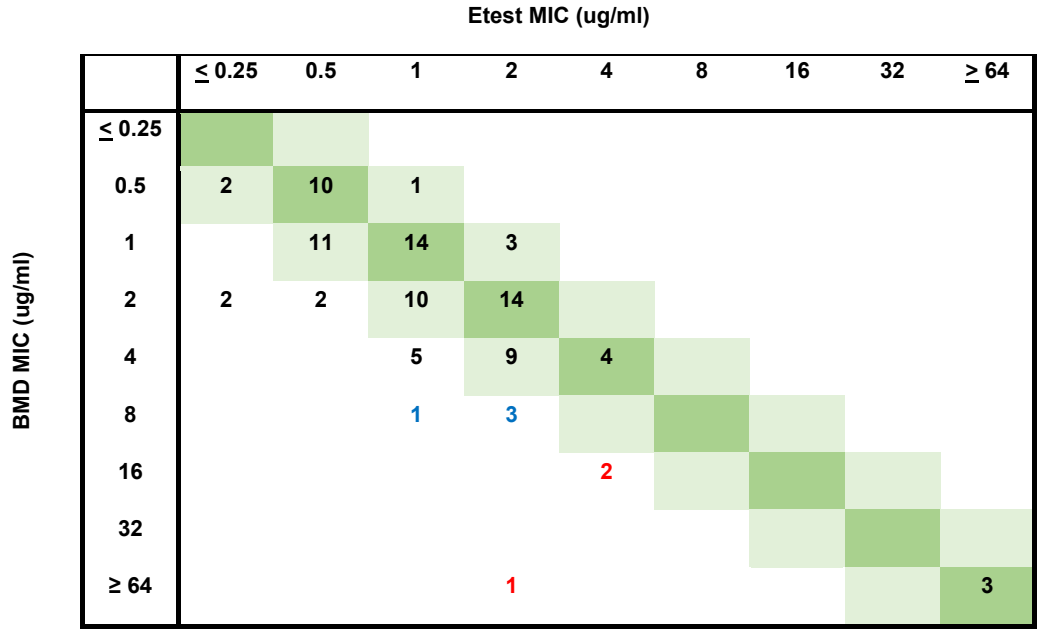
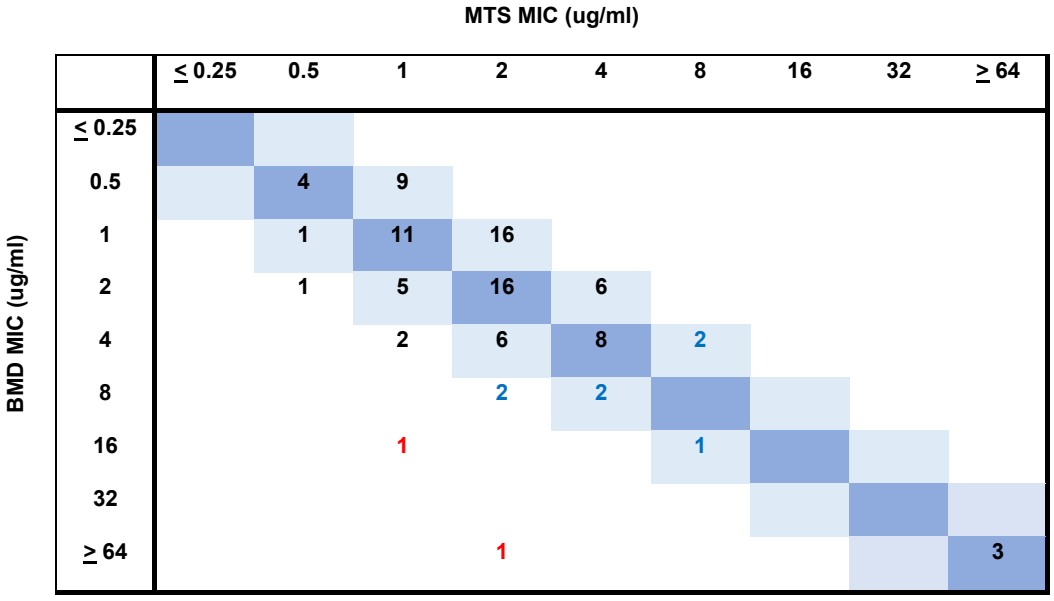
502 **Figure 3.**

503 **A.**



504 **B.**



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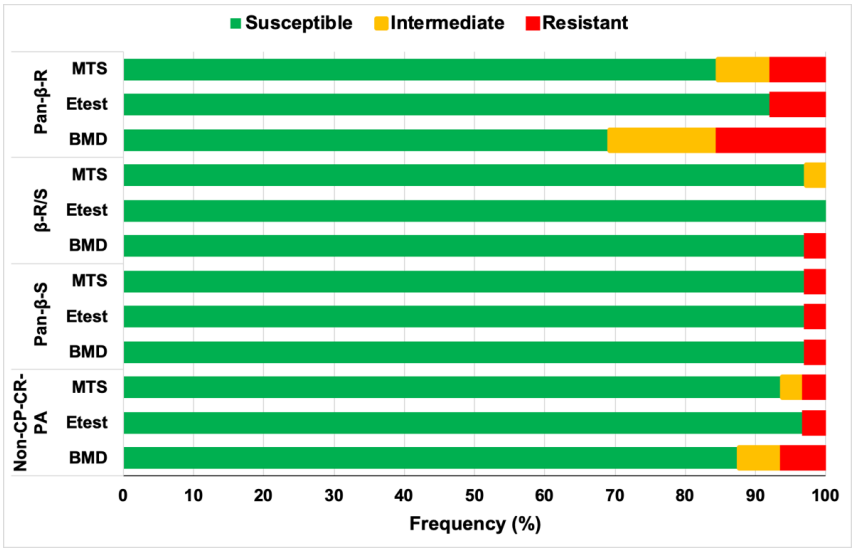
506 **Figure 4.**



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508

509 **Figure 5.**

510 **A.**

	≥ 30	29	28	27	26	25	24	23	22	21	20	19	18	17	≤ 16
≤ 0.25															
0.5	1	2	3	6		1									
1		2	1	5	8	9	2	1							
2			1		4	9	6	6	2						
4			1		3	4	2	3	2	2	1				
8					1	1		1	1						
16											1	1			
32															
≥ 64				1											3

511

512

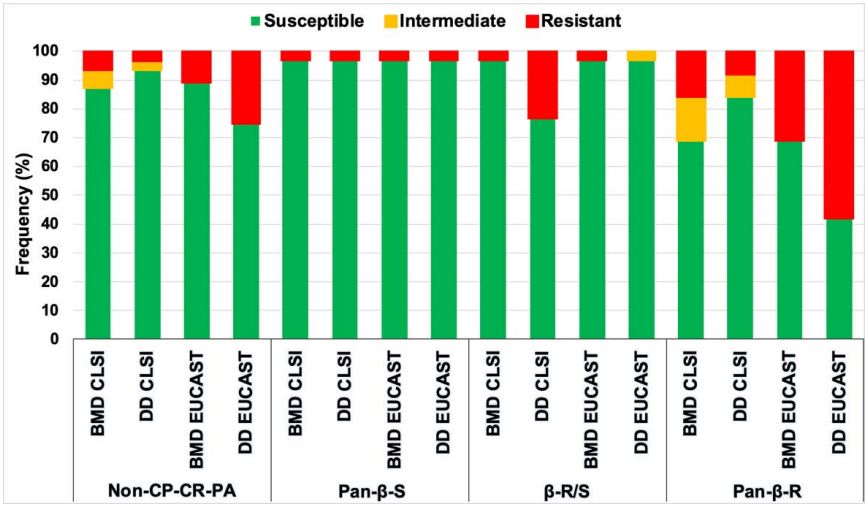
513 **B.**

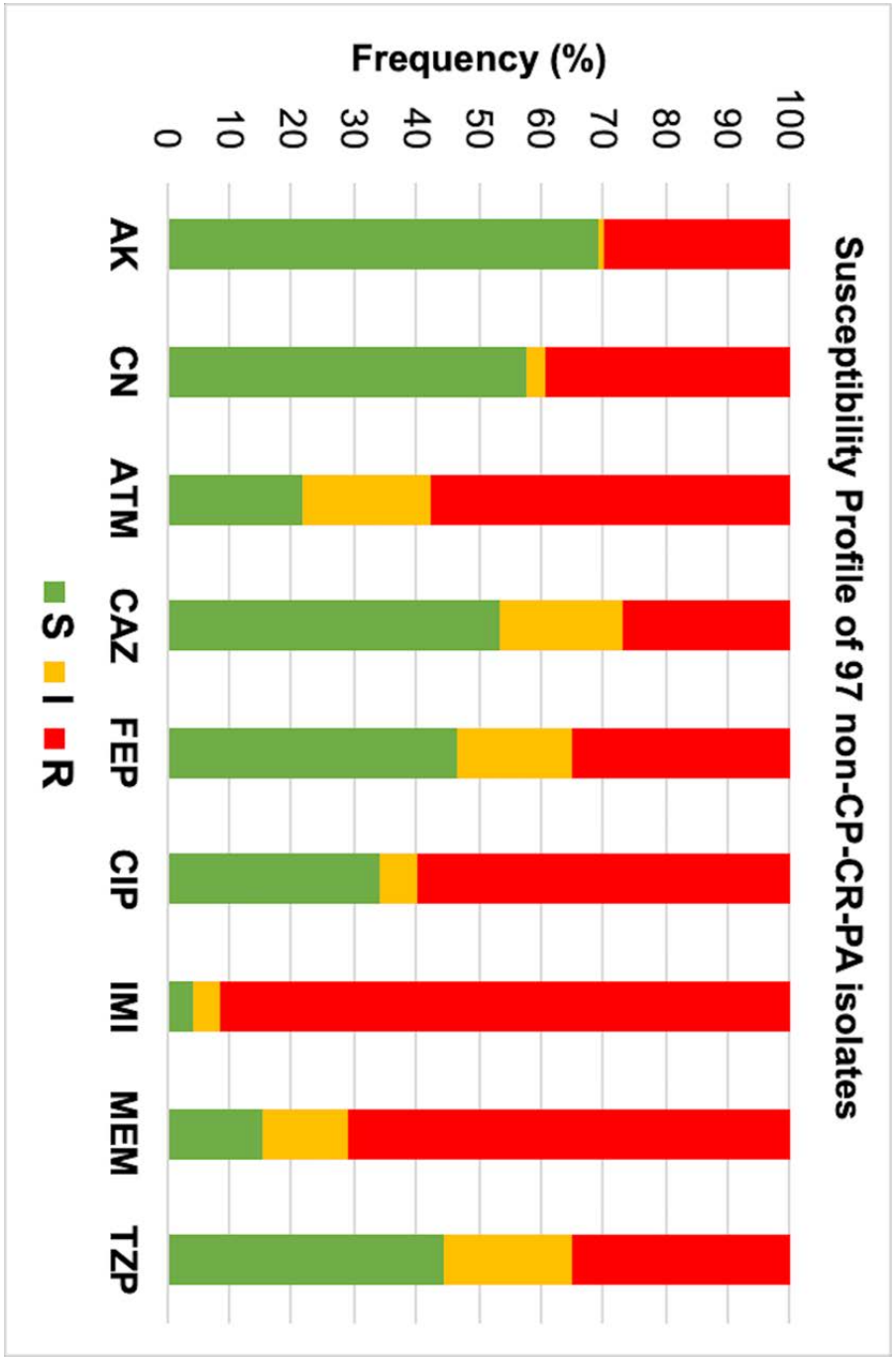
	≥ 30	29	28	27	26	25	24	23	22	21	20	19	18	17	≤ 16
≤ 0.25															
0.5	1	2	3	6		1									
1		2	1	5	8	9	2	1							
2			1		4	9	6	6	2						
4			1		3	4	2	3	2	2	1				
8					1	1		1	1						
16											1	1			
32															
≥ 64				1											3

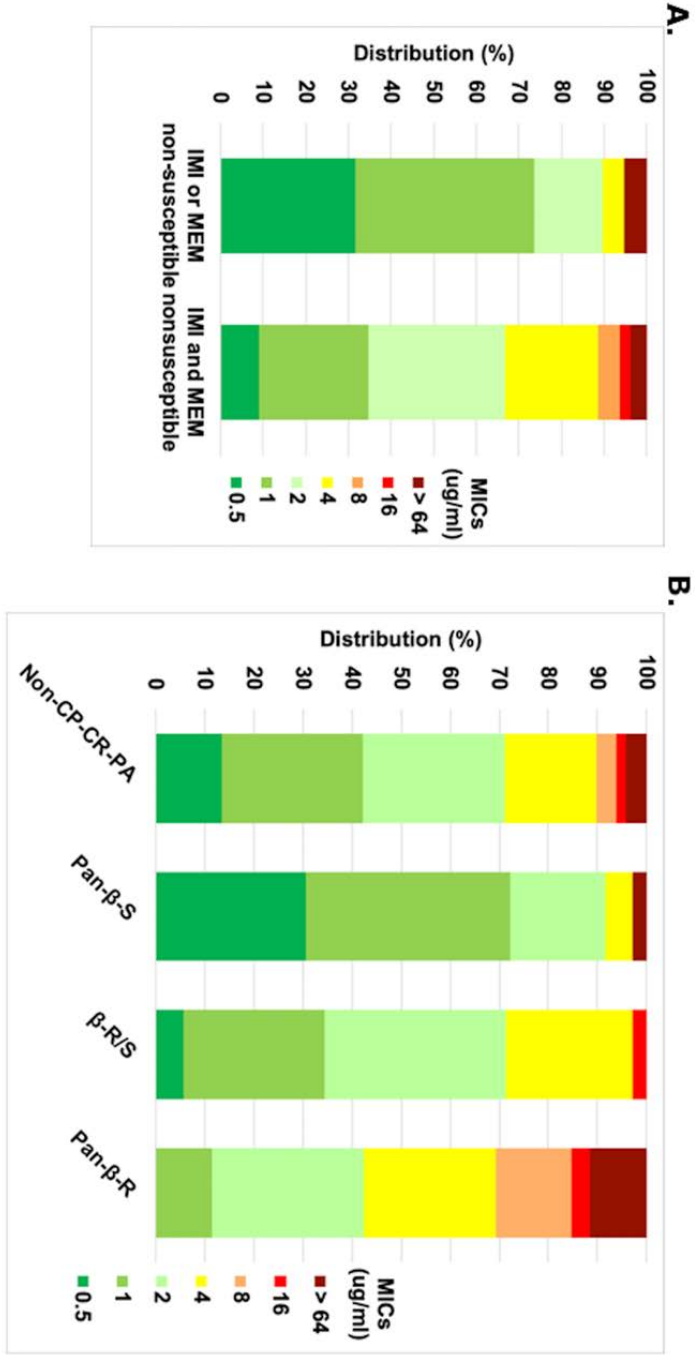
514

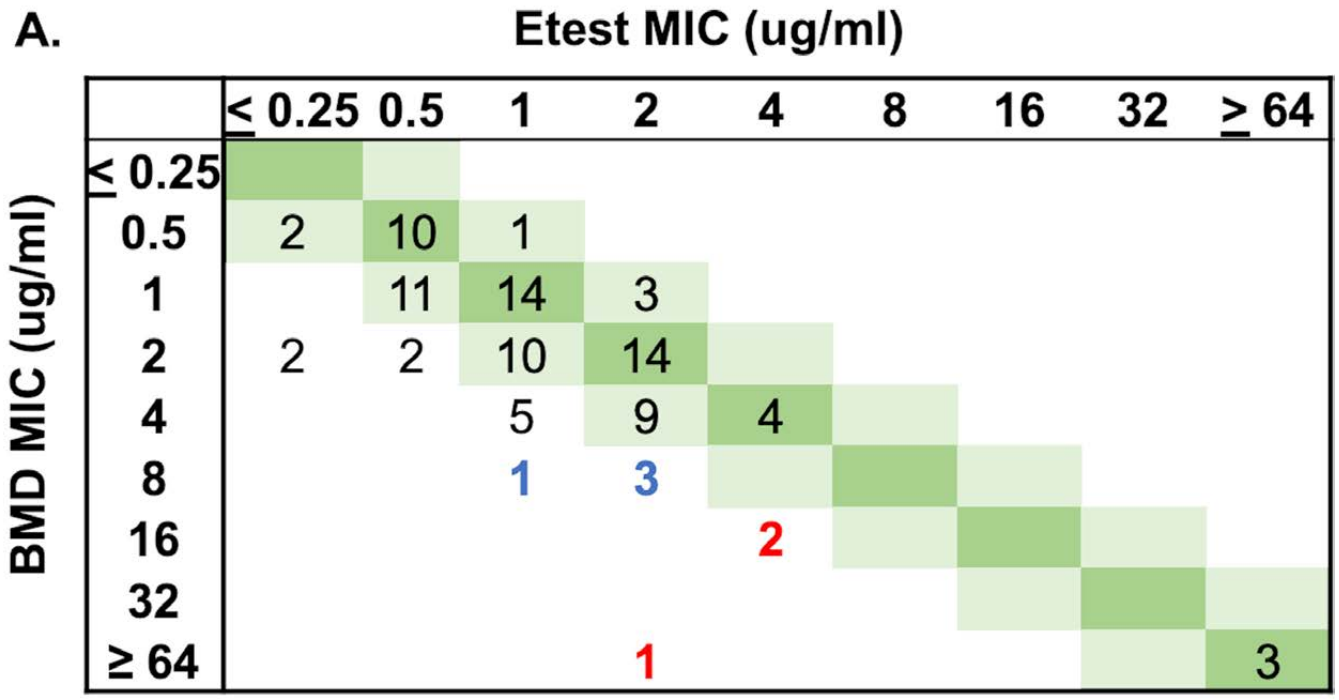
515 **Figure 6.**

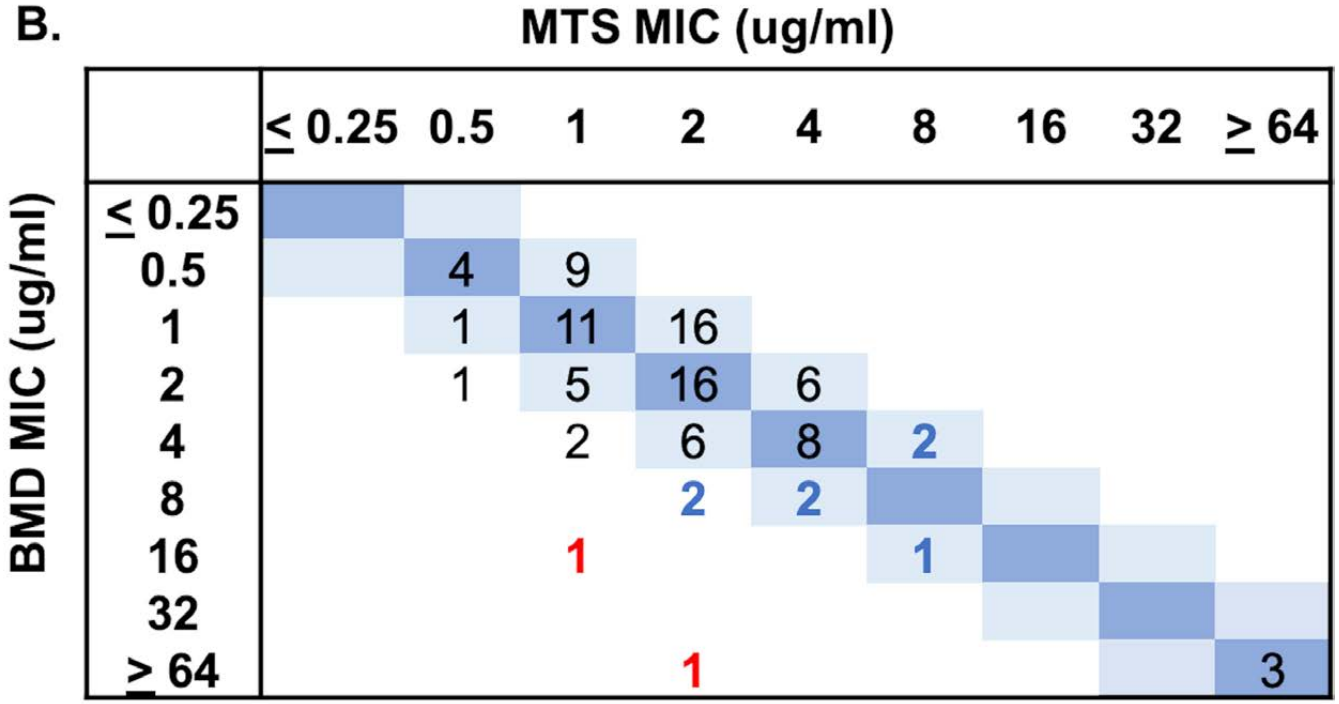
516

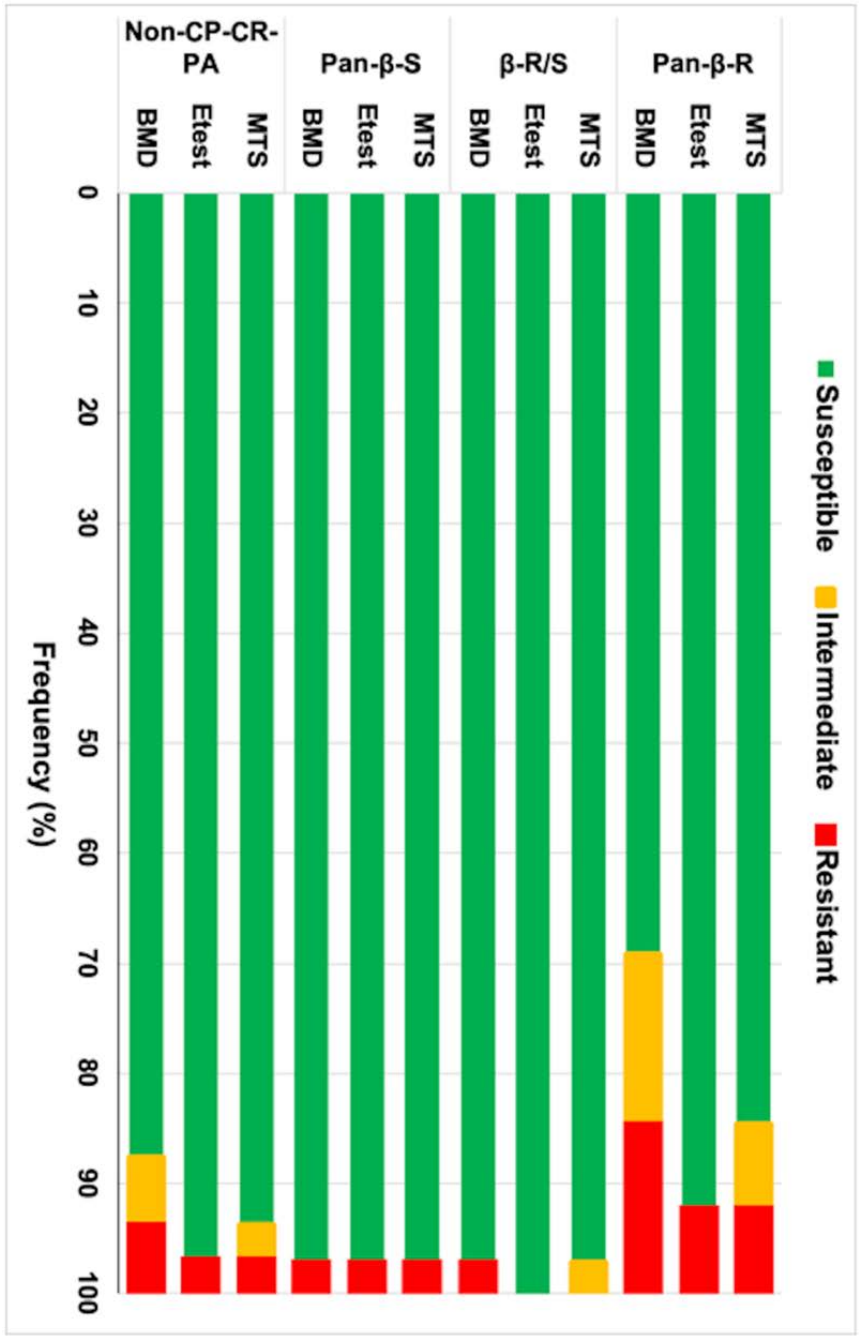












A. DD zone diameter (mm), EUCAST breakpoints

BMD MIC (ug/ml)	≥ 30	29	28	27	26	25	24	23	22	21	20	19	18	17	≤ 16
≤ 0.25															
0.5	1	2	3	6		1									
1		2	1	5	8	9	2	1							
2			1		4	9	6	6	2						
4			1		3	4	2	3	2	2	1				
8					1	1		1	1						
16											1	1			
32															
≥ 64				1											3

B. DD zone diameter (mm), CLSI breakpoints

BMD MIC (ug/ml)	≥ 30	29	28	27	26	25	24	23	22	21	20	19	18	17	≤ 16
≤ 0.25															
0.5	1	2	3	6		1									
1		2	1	5	8	9	2	1							
2			1		4	9	6	6	2						
4			1		3	4	2	3	2	2	1				
8					1	1		1	1						
16											1	1			
32															
≥ 64				1											3