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Agar Disk Diffusion Susceptibility Characteristics of Azlocillin, Carbenicillin, Mezlocillin, Piperacillin, and Ticarcillin

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The agar disk diffusion susceptibility of *Enterobacteriaceae* to mezlocillin and piperacillin was correlated with agar minimal inhibitory concentrations and compared with the susceptibility to carbenicillin. The agar disk susceptibility of *Pseudomonas aeruginosa* to azlocillin, mezlocillin, and piperacillin was correlated with agar minimal inhibitory concentrations and compared with the susceptibility to carbenicillin and ticarcillin. Criteria are offered for the zones of inhibition to provide information about resistant and susceptible isolates that correlate with known serum levels.

A number of penicillins with extended antibacterial spectra have been developed in the past few years. A 100-μg carbenicillin disk has been used to determine the susceptibility of *Enterobacteriaceae* and *Pseudomonas* species to carbenicillin and ticarcillin, the antibiotics currently available for general use in the United States. A 75-μg ticarcillin disk has been evaluated, and zone sizes for resistance have been correlated with the minimal inhibitory concentration (MIC)(9). Two ureido penicillins, mezlocillin and azlocillin, and a piperazine derivative of ampicillin have been shown by several groups to be more active in vitro than carbenicillin against many bacteria (2, 3, 5, 12). To date susceptibility studies correlating zones of inhibition obtained by the disk diffusion method with MICs have not been performed.

For testing *Pseudomonas* and *Enterobacteriaceae* with the 100-μg carbenicillin disk, the National Committee for Clinical Laboratory Standards (NCCLS) recommends a double set of standards for susceptibility (6). Using these standard zone sizes as guidelines, we have developed criteria for zone size interpretation of azlocillin, mezlocillin, piperacillin, and ticarcillin disks. We also have determined the susceptibility of control strains to these agents.

MATERIALS AND METHODS

Isolates. Two hundred fifty-seven recent clinical isolates were studied. All organisms were from clinical specimens of blood, urine, sputum, or wounds of patients admitted to the Columbia-Presbyterian Medical Center, New York, N.Y. The isolated bacteria were inoculated onto brain heart infusion agar (Difco) slants, incubated at 35°C for 18 h, and then maintained at room temperature before testing. The standard reference strains of *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested as internal controls with each set of plates.

Antibiotic susceptibility disks. The antibiotic susceptibility disks used for the study were 100-μg carbenicillin disks (Pfizer), 75-μg ticarcillin disks (BBL Microbiology Systems), 100-μg piperacillin disks (BBL), 30- and 75-μg mezlocillin disks (Difco, Oxoid), and 75-μg azlocillin disks prepared fresh from stock antibiotic (Delbay Laboratory, Bloomfield, N.J.). Antibiotics were provided by their manufacturers: ticarcillin and carbenicillin, Beecham Pharmaceuticals; piperacillin, Lederle Laboratories; azlocillin and mezlocillin, Delbay Laboratories.

Susceptibility tests. MICs were determined by the agar dilution method, utilizing a replicating device which delivered a drop to agar plates (11). More detailed descriptions of the method have been published (9). The standard inoculum used was 10^5 colony-forming units. The MICs and the disk diffusion tests were performed at the same time. Organisms were grown overnight in Mueller-Hinton broth and diluted to the proper inoculum in broth. The inoculum for the disk diffusion was adjusted to a 0.5 MacFarland opacity standard. Both agar dilution plates and disk diffusion plates (Mueller-Hinton agar, BBL) were incubated for 18 h at 35°C.

The breakpoints used for determination of strains susceptible to carbenicillin were based on the NCCLS recommendations (6). *P. aeruginosa* were considered susceptible if the inhibition zone size was ≥17 mm and resistant if the zone size was ≤14 mm. When testing *Enterobacteriaceae* the zone size for susceptibility was ≥23 mm and that for resistance was ≤17 mm. The carbenicillin MICs corresponding to these zone sizes were used as criteria to determine susceptibility and resistance with azlocillin, mezlocillin, piperacillin, and ticarcillin.

The zone sizes were reported to the nearest 0.5 mm.

The zone sizes were plotted against MICs on semilogarithmic paper to produce a scattergram, and regression lines were determined by the method of least squares. All MICs above and below the actual concentrations tested and all disks showing no zone of inhibition were excluded from the calculations.

RESULTS

The in vitro susceptibilities of 257 strains of gram-negative bacilli, as determined by the agar dilution method, are presented in Table 1. Piperacillin inhibited 88% of *P. aeruginosa* at 25 μg/ml; azlocillin, 84%; ticarcillin, 51%; mezlocillin, 49%; and carbenicillin, 20%. *E. coli*, *Proteus mirabilis*, and *Enterobacter* exhibited similar susceptibility to carbenicillin, mezlocillin, and piperacillin, with a biphasic distribution of MICs either very susceptible (≤25 μg/ml) or very resistant (≥400 μg/ml). Only mezlocillin and piperacillin inhibited *Klebsiella*. Table 2 shows the susceptibilities of control strains *E. coli* ATCC 25992 and *P. aeruginosa* ATCC 27853 to the agents.

Zone sizes corresponding to MICs for each agent are shown in Fig. 1. The mathematical analysis (Table 3) showed that in testing the susceptibility of the *Enterobacteriaceae* with the 100-μg carbenicillin disk, the 30- or 75-μg mezlocillin disk, and the 100-μg piperacillin disk, correlations were excellent (r = 0.80 to 0.85). Testing the susceptibility of *P. aeruginosa*, the correlation coefficients were satisfactory for carbenicillin and mezlocillin (r = 0.81) but lower for azlocillin, piperacillin, and ticarcillin (r = 0.60, 0.75, 0.77). This was due to clustering at similar MICs and to the number of organisms with an MIC of 400 μg/ml which had zones of inhibition up to 15 μg/ml.

Testing *P. aeruginosa* with the 100-μg carbenicillin disk and using the NCCLS recommended zone sizes, ≥17 mm for susceptibility and ≤13 mm for resistance, the projection from the regression line would yield MICs of 168 μg/ml as resistant and 84 μg or less per ml as susceptible (Fig. 2). With the 75-μg azlocillin disk, the

TABLE 1. *Comparison of in vitro activity of mezlocillin, piperacillin, carbenicillin, azlocillin, and ticarcillin*

Organism	No. of strains	Agent	Cumulative MIC (μg/ml)									
			≤0.8	1.6	3.1	6.2	12.5	25	50	100	200	≥400
E. coli	40	Mezlocillin	18	28	43	58		61				100
		Piperacillin	25	38	51	61					64	100
		Carbenicillin		10	28	45	55	60				100
K. pneumoniae	29	Mezlocillin			7	17	28	38	45	52		100
		Piperacillin		3	14	28		38	48	52		100
		Carbenicillin							7	14	17	100
P. mirabilis	13	Mezlocillin	62	85								100
		Piperacillin	85									100
		Carbenicillin	85									100
Proteus (indole-positive)	17	Mezlocillin	18	53	65		71	88				100
		Piperacillin	41	59	65	76	82	88				100
		Carbenicillin	29	47	53	59	71	76			82	100
S. marcescens	16	Mezlocillin				19					32	100
		Piperacillin		13	19						25	100
		Carbenicillin				19						100
Acinetobacter	11	Mezlocillin	9		18			27	54	64	73	100
		Piperacillin	9	18	27	36		63		72		100
		Carbenicillin		9			36		55			100
Enterobacter	27	Mezlocillin		11	48	70	85	89		96		100
		Piperacillin	15	26	70	85	93		96			100
		Carbenicillin		7	41	59	74	78	89		96	100
Citrobacter	7	Mezlocillin			29	57	71			100		
		Piperacillin			29	43	58	86				100
		Carbenicillin							29	57	71	100
P. aeruginosa	85	Mezlocillin		2	5		19	49	76	91	94	100
		Piperacillin	4	8	35	59	79	88	91	94	97	100
		Carbenicillin	1		4	5	6	20	53	69	84	100
		Azlocillin	1	2	12	41	69	84	91	94	97	100
		Ticarcillin	1	4		9	22	51	69	75	84	100
Other *Pseudomonas* sp.	12	Mezlocillin					17	25	33	67	92	100
		Piperacillin						25		50	83	100
		Carbenicillin			8	17		42	50	58	83	100
		Azlocillin				8			17	50	67	100
		Ticarcillin					8		17	42	50	100

TABLE 2. *Antibiotic activity against control strains*[a]

Antimicrobial agent	Disk content (μg)	Zone diam of inhibition (mm)				MIC (μg/ml)			
		E. coli (ATCC 25922)		*P. aeruginosa* (ATCC 27853)		*E. coli* (ATCC 25922)		*P. aeruginosa* (ATCC 27853)	
		Mean[b]	Range	Mean	Range	Median[c]	Range	Median	Range
Azlocillin	75	22.0	20–25	27.8	27–29	6	3–6	5	3–6
Carbenicillin	100	26.2	25–29	22.4	20–24	6	3–7	50	25–50
Mezlocillin	75	25.5	24.5–26	22.5	22–25	1.5	1–3	20	12–25
Mezlocillin	30	22.0	20–24	17.6	17–19				
Piperacillin	100	27.0	25–30	30.6	29–32	3	1–3	3	2–4
Ticarcillin	75	27.0	25–28	25.1	24–26	3	2–3.5	20	12.5–25

[a] Tested on Mueller-Hinton agar; MICs are tested in arithmetic progression of 0.5 μg/ml.
[b] Means are based on 10 replicate determinations.
[c] Medians are based on the inhibition of 50% or more of 16 replicate determinations.

regression line crossed the "no zone" at an MIC of 115 μg/ml, too low to determine resistance. Testing susceptibility of the *P. aeruginosa* with the 75-μg mezlocillin disk, the 100-μg piperacillin disk, and the 75-μg ticarcillin disk, the zone sizes for susceptibility were, respectively, ≥15, ≥17, and ≥17 mm, whereas the zone sizes for resistance were ≤12 mm for mezlocillin, ≤13 mm for piperacillin, and ≤13 mm for ticarcillin, if one used the criteria of ≥ 200 μg/ml for resistance and ≤100 μg/ml for susceptibility.

For testing *Enterobacteriaceae* with the 100-μg carbenicillin disk, the NCCLS recommends a zone size of ≥23 mm for susceptibility and ≤17 mm for resistance. Using a projection from the regression line, an MIC of 50 μg/ml was resistant and one of 12.5 μg or less per ml was susceptible (Fig. 1). These MICs were the criteria used to determine the susceptibility and resistance zone sizes with mezlocillin and piperacillin. With the 75- and 30-μg mezlocillin disks and the 100-μg piperacillin disk, the zone sizes for susceptibility were, respectively ≥22, ≥19, and ≥22 mm, whereas the zone sizes for resistance were: mezlocillin (75-μg disk), ≤17 mm; mezlocillin (30-μg disk), ≤14 mm; and piperacillin, ≤18 mm.

There were few *P. aeruginosa* resistant (MIC, 168 μg/ml) to mezlocillin (10.6%) and piperacillin (5.9%), so the evaluation of disk zone sizes for resistance or intermediate susceptibility was based on projection of the regression lines. Therefore, the recommended zone sizes for susceptibility (Table 4) delineated strains susceptible (MIC, ≤84 μg/ml) to piperacillin (77 of 81) and mezlocillin (66 of 72) well. Looking for either false-positive (susceptible with disk and resistant by MIC) or false-negative (resistant with disk and susceptible by MIC) readings with the proposed zone sizes, the predictability of the carbenicillin disk was 94.1%, that of mezlocillin was 94.1%, and that of piperacillin was 96.5%. The frequency of false-positive readings with the carbenicillin disk was 3.5%, that with mezlocillin was 2.4%, that with piperacillin was 3.5%, and that with ticarcillin was 4.7%.

DISCUSSION

The in vitro activities of carbenicillin, ticarcillin, azlocillin, mezlocillin, and piperacillin have been reported previously (2, 3, 5, 12), and this study confirms the same range of activity.

There are no official criteria for the zone sizes obtained with ticarcillin disks (75 μg), although the antibiotic is in wide clinical use. Pharmacological studies of mezlocillin (4, 8), azlocillin (1), and piperacillin (S. J. Pancoast, E. Francke, and H. C. Neu, manuscript in preparation) have shown that these new agents produce serum and tissue levels similar to those achieved with carbenicillin and ticarcillin (7).

To designate susceptible, intermediate, and resistant organisms, different parameters may be used. In general, the MIC determined as the "breakpoint" for susceptibility should be lower than the achievable blood level. Using a disk, we should be able to discriminate between different MICs so that very few organisms would be judged either inappropriately susceptible or resistant to the agent, considering the achievable serum levels.

In this study the susceptibilities of *P. aeruginosa* and *Enterobacteriaceae* were set with different MICs, 100 and 12.5 μg/ml, respectively, because there is a continuum of susceptibility of *Pseudomonas* to these penicillins whereas the *Enterobacteriaceae* tend to fall into susceptible or resistant categories by virtue of the types of β-lactamases or cell wall they possess (2, 3).

Testing *P. aeruginosa* with a 75-μg ticarcillin disk, our zone sizes of ≥17 mm for susceptibility and ≤13 mm for resistance differ slightly from the manufacturers' proposed zone sizes of ≥16 and ≤11 mm, respectively. However, the predictability of zone sizes with our criteria is the

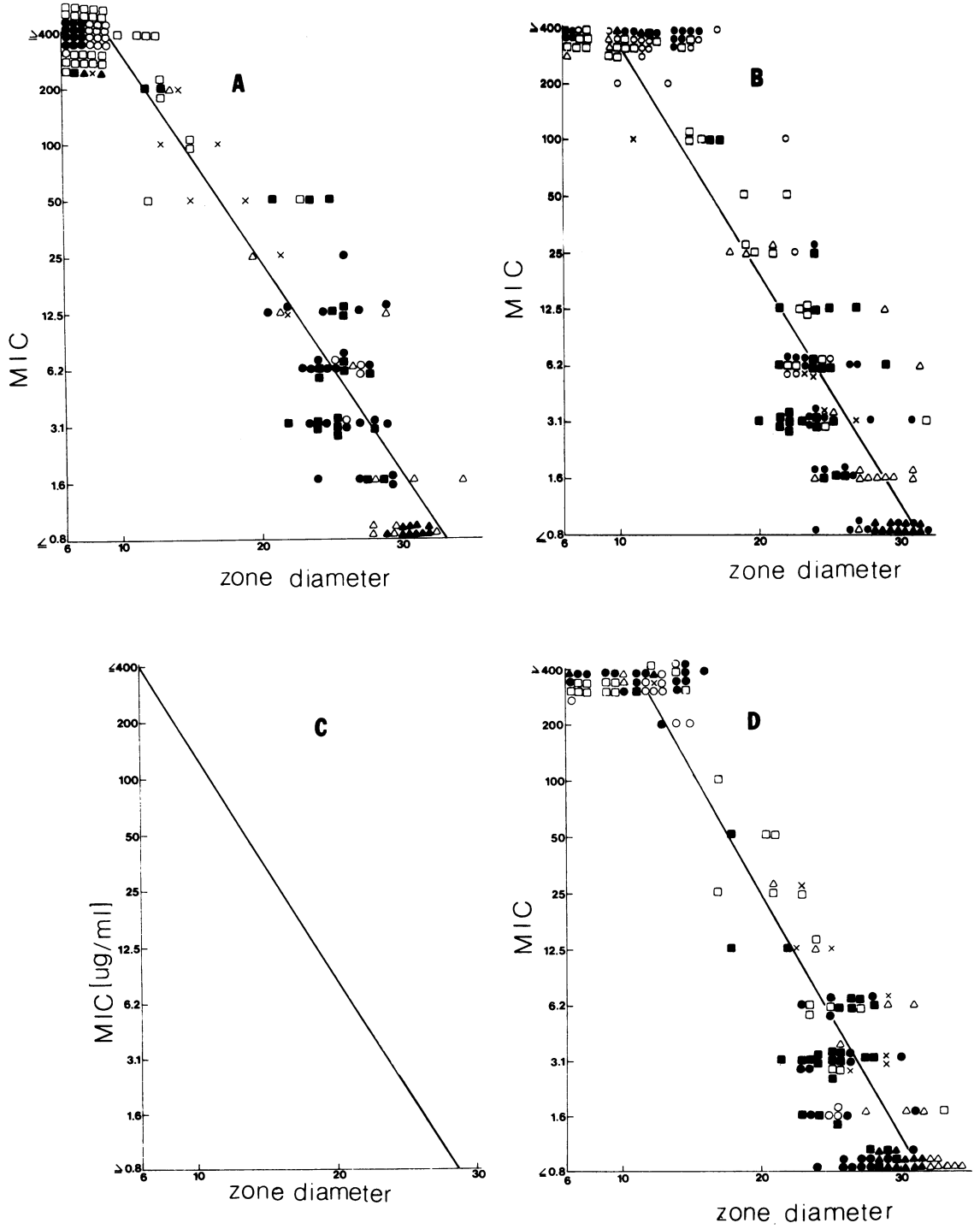

FIG. 1. *Correlation between inhibition zones and the agar MICs for Enterobacteriaceae with: (A) carbenicillin, 100 μg; (B) mezlocillin, 75 μg; (C) mezlocillin, 30 μg; (D) piperacillin, 100 μg. Symbols: E. coli* (●); *Serratia* (○); *P. mirabilis* (▲); *Proteus, indole-positive* (△); *Enterobacter* (■); *Klebsiella* (□); *Citrobacter* (×).

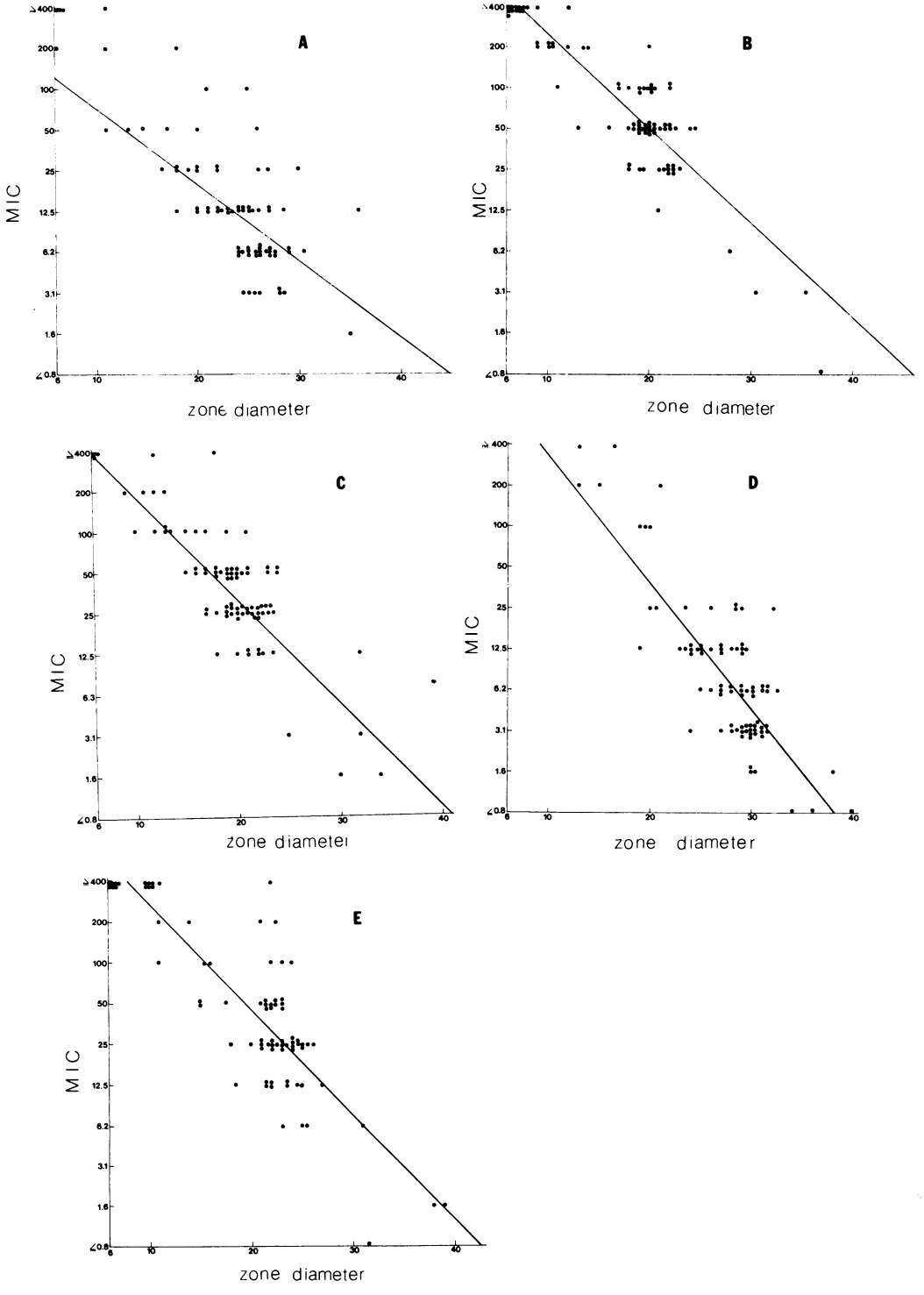

FIG. 2. *Correlation between inhibition zones and agar MICs for P. aeruginosa with: (A) azlocillin, 75 μg; (B) carbenicillin, 100 μg; (C) mezlocillin, 75 μg; (D) piperacillin, 100 μg; (E) ticarcillin, 75 μg.*

TABLE 3. *Mathematical analysis of susceptibility data*

Antibiotic	Disk content (μg)	Organism	No. tested	Correlation coefficient (*r*)	Slope (*m*)	Y intercept[a]	No zone (μg/ml)
Azlocillin	75	*P. aeruginosa*	78	0.6041	−0.1835	7.9544	115.30
Carbenicillin	100	*P. aeruginosa*	70	0.8160	−0.2303	10.2421	464.36
Mezlocillin	75	*P. aeruginosa*	79	0.8135	−0.2489	9.9677	354.38
Piperacillin	100	*P. aeruginosa*	79	0.7564	−0.3069	11.4384	770.17
Ticarcillin	75	*P. aeruginosa*	77	0.7781	−0.2590	10.6913	563.82
Carbenicillin	100	*Enterobacteriaceae*	75	0.8531	−0.3671	11.9229	842.79
Mezlocillin	75	*Enterobacteriaceae*	90	0.8084	−0.3935	12.1008	854.55
Mezlocillin	30	*Enterobacteriaceae*	87	0.8017	−0.3913	10.8872	369.42
Piperacillin	100	*Enterobacteriaceae*	75	0.8208	−0.4223	13.0325	1,437.11

[a] Expressed in $\log_2$ scale.

TABLE 4. *Zone size interpretative chart and predictability*

Antibiotic	Potency (μg)	Organism	Diam of zone of inhibition (mm)			Predictability (%)
			Resistant	Intermediate	Susceptible	
Carbenicillin	100	*P. aeruginosa*	≤13	14–16	≥17	94.1
Mezlocillin	75	*P. aeruginosa*	≤12	13–14	≥15	94.1
Piperacillin	100	*P. aeruginosa*	≤13	14–16	≥17	86.5
Ticarcillin	75	*P. aeruginosa*	≤13	14–16	≥17	94.1
Carbenicillin	100	*Enterobacteriaceae*	≤17	18–22	≥23	96.7
Mezlocillin	75	*Enterobacteriaceae*	≤17	18–21	≥22	98
Mezlocillin	30	*Enterobacteriaceae*	≤14	15–18	≥19	98
Piperacillin	100	*Enterobacteriaceae*	≤18	19–21	≥22	98

same as that with the manufacturers' criteria (94.1%).

Testing susceptibility of *Enterobacteriaceae* with 75-μg and 30-μg mezlocillin disks yielded excellent correlation (r = 0.808 and 0.801), and the predictability of zone sizes was satisfactory for both disks (98%). The zone size with the 75-μg mezlocillin disk was easier to read and was closer to the zone size achieved with the 100-μg carbenicillin disk. The 100-μg piperacillin disk produced a correlation coefficient of 0.82 and a predictability of the zone sizes of 98%.

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