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Letters to the Editor

Can Etest Be Used To Determine *Vibrio cholerae* Susceptibility to Erythromycin?

Vibrio cholerae causes cholera, which, in severe cases and without treatment, has a mortality rate as high as 60% (5). In severe cases, antibiotic therapy is used to reduce the duration of diarrhea and excretion of *V. cholerae*, thus controlling the spread of the disease. Various antibiotics have been used for treatment. For pregnant women and children, erythromycin or trimethoprim-sulfamethoxazole are preferred choices (4). The seventh cholera pandemic spread to Latin America in 1991 (1). At the beginning, strains obtained from Peru were susceptible to the recommended antibiotics, but soon, Ecuador reported multiple resistant vibrios (4). Therefore, monitoring the susceptibilities of *V. cholerae* to recommended therapies is important.

Although the disk diffusion method is suitable for susceptibility surveillance, erythromycin disk diffusion results do not correlate well with MIC-based dilution methods (2). Therefore, we explored the use of the Etest MIC method to determine the erythromycin susceptibility of *V. cholerae*.

To determine the interlaboratory reproducibility of Etest results, five laboratories used Etest in parallel with agar dilution (4) to test 18 *V. cholerae* clinical strains (Table 1). *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains. Four laboratories performed the tests twice, and one laboratory performed it once. When the Etest MIC did not correspond to the standard twofold dilution value, it was rounded up to the next higher dilution.

As shown in Table 1, Etest MICs showed satisfactory interlaboratory agreement with results for 17 of 18 strains (94.4%) being within 1 dilution and the result for the other strain (98-0965) being within 2 dilutions. For agar dilution, MIC results for 14 of 18 strains (77.8%) were within 1 dilution and those for the 4 remaining strains were within 2 dilutions. When Etest values were compared to those of agar dilution, 13% were identical, 67.3% were within 1 dilution, 98.1% were within 2 dilutions, and three results were within 3 dilutions.

Currently, no erythromycin interpretive breakpoints for *V. cholerae* exist (3). However, the bimodal distribution of erythromycin MICs for this study's strains showed that for the susceptible population the modal MIC was 1 to 2 μg/ml by Etest and 2 to 8 μg/ml by agar dilution (Table 1). Etest results gave a wider MIC "corridor" between the two populations. For *V. cholerae*, 16 μg/ml could be used to define resistant phenotypes (Table 1). More isolates for which MICs are around 4 to 8 μg/ml are needed to distinguish intermediate from resistant phenotypes. The Etest MIC method was found to be reproducible and comprises an economical alternative for testing *V. cholerae* in laboratories with infrequent isolation rates. It is also a valuable tool for resistance surveillance on a national or international scale since exact MICs across 15 dilutions will allow both low- and high-level resistance to be detected.

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TABLE 1. Erythromycin agar dilution and Etest MIC ranges for 18 *V. cholerae* strains and 2 quality control strains[a]

Strain	Agar dilution		Etest	
	Range (μg/ml)	Mode	Range (μg/ml)	Mode
97-0016	2.0–4.0	2.0	0.75–2.0	1.0
97-0027	16.0–64.0	32.0	48.0–128.0	48.0
97-0052	2.0–4.0	2.0	0.75–2.0	1.0
97-0256	16.0–64.0	32.0	32.0–64.0	32.0, 64.0
98-0876	2.0–4.0	2.0	0.75–2.0	1.0
98-0883	2.0–4.0	4.0	0.75–2.0	1.0
98-0884	2.0–4.0	2.0	0.75–2.0	1.0
98-0886	2.0–4.0	4.0	0.75–2.0	1.0
98-0944	4.0–8.0	8.0	1.5–3.0	2.0
98-0965	2.0–4.0	2.0	0.5–2.0	0.75
98-0971	2.0–4.0	2.0	0.75–2.0	1.0
98-1106	2.0–4.0	2.0	0.75–1.5	1.0
98-1127	2.0–4.0	2.0	1.0	1.0
98-1129	2.0–4.0	2.0	0.75–1.5	1.0
98-1130	2.0–8.0	2.0, 4.0	1.0–2.0	1.5
98-1186	2.0–8.0	2.0	1.0–2.0	1.0
98-1193	2.0–4.0	4.0	0.75–1.5	1.0
98-1242	2.0–4.0	2.0	0.38–1.0	1.0
E. faecalis ATCC 29212	1.0–2.0	2.0	1.0–4.0	4.0
S. aureus ATCC 29213	0.25–0.5	0.25	0.125–0.38	0.125

[a] Agar dilution and Etest MIC ranges are within 2 dilutions from mode.

REFERENCES

1. **Blake, P. A.** 1994. Historical perspectives on pandemic cholera, p. 293–295. *In* I. K. Wachsmuth, P. A. Blake, and Ø. Olsvik (ed.), *Vibrio cholerae* and cholera: molecular to global perspectives. ASM Press, Washington, D.C.
2. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial susceptibility testing, 12th informational supplement. M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
3. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 5th ed. M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
4. **Sack, D. A., C. Lyke, C. McLaughlin, and V. Suwanvanichkij.** 2001. Antimicrobial resistance in shigellosis, cholera and campylobacteriosis (WHO/CDS/CSR/DRS/2001.8), p. 13. World Health Organization, Geneva, Switzerland.
5. **Virella, G., and M. G. Schmidt.** 1997. Gram-negative rods II: Enterobacteriaceae and other enteropathogenic gram-negative rods, p. 141–157. *In* G. Virella (ed.), Microbiology and infectious diseases. The Williams & Wilkins Co., Baltimore, Md.

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