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Evaluation of DNA Probes for Specific Detection of *Vibrio cholerae* O139 Bengal

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Two DNA probes, 2R1 and 2R3, prepared from a region in the chromosome specific for the lipopolysaccharide O side chains of *Vibrio cholerae* O139 (M. K. Waldor and J. J. Mekalanos, Lancet 343:1366, 1994) were examined for their specificity and sensitivity. Both probes did not hybridize with any strain of *V. cholerae* belonging to serogroups other than O139 and to any of the other species examined belonging to the family *Vibrionaceae*. Among the 126 strains of *V. cholerae* O139 examined, probe 2R1 hybridized with 125 strains while probe 2R3 hybridized with all 126 strains. Both probes were found to be highly specific and sensitive and can be used for the specific identification of *V. cholerae* O139.

With the emergence of *Vibrio cholerae* O139 serogroup (1, 9) and with the recognition that the O139 serogroup causes cholera indistinguishable from that caused by the O1 serogroup (2, 5), it has become necessary to make a quick and definitive diagnosis of cholera caused by the O139 serogroup. Because of the overall similarity in the biochemical and physiological characteristics of *V. cholerae* O1 and O139 (8), the laboratory diagnosis of the O139 serogroup is essentially similar to that of the O1 serogroup (7). Specific absorbed antiserum should, however, be used for the serological confirmation of the O139 serogroup. The O139 antiserum prepared by hyperimmunizing rabbits with heat-killed whole cells of reference strain O139 (ATCC 51394) must be absorbed with R strain (strain number CA 385) of *V. cholerae* and with reference strains 169-68 and 490-93, representing serogroups O22 and O155 of *V. cholerae*, to remove cross-reacting agglutinins (10, 11).

By transposon insertion mutagenesis, Waldor and Mekalanos (12) have recently demonstrated that O139 transposon mutants which did not agglutinate with anti-O139 antiserum lacked lipopolysaccharide O side chains, indicating that the genes for the biosynthesis of these two structures are common and *V. cholerae* O139 must have acquired unique DNA during evolution. The potential of the O139-specific DNA fragment identified by the above investigators to be a useful diagnostic tool was examined in this study. Our rationale was that if the probes were sensitive and specific, such probes could be used as an adjunct to serological methods for the laboratory diagnosis of *V. cholerae* O139.

To examine the sensitivity and specificity of two DNA probes, 2R1 and 2R3, for *V. cholerae* O139, we examined a total of 643 strains belonging to a variety of species in the family *Vibrionaceae* as listed in Table 1. This group included the reference strains of all 155 currently documented serogroups of *V. cholerae* (10, 11), including *V. cholerae* O139 Bengal (ATCC 51394), and also included type strains of the following *Vibrio* species: *V. natriegens* (ATCC 14018), *V. vulnificus* (ATCC 27561), *V. fluvialis* (ATCC 33809), *V. furnissii* (ATCC 35016), and *V. hollisae* (ATCC 33564). The *V. cholerae* reference strains were from the National Institute of Health, Tokyo, Japan; the clinical strains of *V. cholerae* O1 and O139 were from the culture collection of the National Institute of Cholera and Enteric Diseases, Calcutta, India, and from the Department of Microbiology, Kyoto University, Kyoto, Japan; and the other species belonging to the family *Vibrionaceae* were from the culture collection of the Department of Infectious Diseases Research, National Children's Medical Research Center, Tokyo, Japan. Colony blots of the strains listed in Table 1 were prepared on autoclaved nitrocellulose filters with grids (Schleicher and Schuell, Dassel, Germany).

The O139-specific DNA probes, 2R1 and 2R3 (a gift from M. K. Waldor and J. J. Mekalanos), were derived by cloning (via inverse PCR) the DNA adjacent to Tn*5lac* insertions in MO10 (an O139 strain from the Madras outbreak) that rendered the strain O139 negative, as confirmed by slide agglutination with specific antiserum (12). Probe 2R1 has only about 100 bp of O139 DNA, and probe 2R3 has about 1,000 bp of O139-specific DNA. Both the 2R1 and 2R3 fragments were separated from their vectors (pCRII-I3 and pCRII-A3, respectively) by *Eco*RI digestion and purified after preparative gel electrophoresis by the Qiagen plasmid purification procedure (Qiagen Inc., Chatsworth, Calif.). The probes were labeled with 50 μCi of [α-^{32}P]dATP (Amersham International plc., Amersham, United Kingdom) with the Multiprime DNA labeling system (Amersham International) according to the manufacturer's specifications. Hybridization was performed at 37°C in 50% formamide, 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5 mM EDTA, 1× Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), and 100 μg of salmon sperm DNA per ml, followed by a high stringency wash at 65°C in 5× SSC–0.1% SDS (6).

A total of 643 strains from 14 species of the family *Vibrion-*

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TABLE 1. Results of colony hybridizaton with *V. cholerae* O139-specific DNA probes

Vibrio strain or species	No. examined	No. of strains which hybridized with:	
		2R1	2R3
V. cholerae reference strains	158	0	0
V. cholerae O1 (wild-type clinical strains)	247	0	0
V. cholerae rough strains	2	0	0
V. cholerae O139 reference strain	1	1	1
V. cholerae O139 (wild-type clinical strains)	125	124	125
V. parahaemolyticus	22	0	0
V. fluvialis	13	0	0
V. anguillarum	2	0	0
V. mimicus	20	0	0
V. alginolyticus	11	0	0
V. vulnificus	16	0	0
V. metschnikovii	6	0	0
V. nereis	1	0	0
V. natriegens	1	0	0
V. harveyi	2	0	0
V. furnissii	6	0	0
V. hollisae	5	0	0
V. damsela	5	0	0

aceae were examined by O139-specific DNA probes 2R1 and 2R3. Neither probe hybridized with any *V. cholerae* strains belonging to serogroups other than O139, nor did they hybridize with any of the other species in the family *Vibrionaceae*. Both probes were specific. Among the 126 strains of *V. cholerae* O139 examined, including the reference strain ATCC 51394, probe 2R1 hybridized with 125 strains while probe 2R3 hybridized with all 126 strains of O139 examined. The O139 strain (R2-359) which did not hybridize with probe 2R1 was isolated from Malaysia. Subsequent studies using pulsed-field gel electrophoresis showed that the restriction fragment length polymorphism of *Not*I-digested genomic DNA of strain R2-359 was slightly different from the other strains of *V. cholerae* O139, indicating genetic polymorphism in this strain (4). Since probe 2R1 contained only about 100 bp of O139-specific DNA fragment, it appears that an alteration has taken place in this region of the DNA fragment, preventing probe 2R1 from hybridizing with this strain.

Recently, Comstock et al. (3) identified a chromosomal region of at least 11 kb in *V. cholerae* O139, as defined by three Tn*phoA* mutations, that is required for the expression of both the polysaccharide capsule and the distinct O139 O antigens. However, in contrast to our data, DNA probes for this region did not hybridize with O1 *V. cholerae* but did react with other vibrios. These results imply that the DNA fragment from which the 2R1 and 2R3 probes were constructed are associated only with the O139-associated genetic element and therefore did not hybridize with any other species of vibrios or with any other serogroups of *V. cholerae*. This finding again attests to the specificity of these two O139-specific DNA probes and reaffirms their suitability for use as a diagnostic tool.

Serogrouping with well-absorbed antiserum is undoubtedly the simplest means of identifying the O139 serogroup. However, under certain circumstances, such as when processing multiple strains during an outbreak or in epidemiological studies when a large number of isolates is to be examined, the O139-specific DNA probes would be of great use. In addition, the O139 DNA probes evaluated in this study would be of great help in searching for the O139 serogroup among the multitude of naturally occurring non-O1 serogroups of *V. cholerae* in environmental studies, since this would entail screening several thousand strains to understand the ecology of the O139 serogroup in natural aquatic environs. The α-^{32}P label used in this study is likely to limit the use of the probe to laboratories with radioisotope facilities. However, this limitation can be overcome by the use of several nonradioisotope labeling systems currently available.

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