

ISOLATION OF UREASE-POSITIVE *VIBRIO PARAHAEMOLYTICUS* FROM DIARRHEAL PATIENTS IN NORTHEAST BRAZIL

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SUMMARY

Of 21 human fecal strains of *Vibrio parahaemolyticus*, isolated on the Northeast Coast of Brazil, eight (38%) were urease positive. Most of these strains, in contrast to the urease-negative ones, did not produce the hemolysin responsible for the Kanagawa phenomenon.

KEY WORDS: *Vibrio parahaemolyticus*; *Vibrio* diarrhea; *Vibrio* urease; Kanagawa phenomenon.

Vibrio parahaemolyticus has long been recognized as an important cause of seafood linked gastroenteritis in humans⁵. The microorganism was thoroughly studied from the biochemical viewpoint and urease negativity is one of its accepted properties¹². In the last years, however, gastroenteritis associated with urease-positive strains of *V. parahaemolyticus* are being increasingly identified^{6, 7, 9, 10, 11}. In this vein, of particular interest are the reports on the recent emergence of urease-positive strains as the predominant biotype of *V. parahaemolyticus* in the Pacific Northwest and on the West Coast of the United States and Mexico¹.

In Recife, a tropical city situated in Brazil Northeast, we have recently observed that 38% of *V. parahaemolyticus* strains recovered from cases of human gastroenteritis were urease positive. Now, we present information on serological characteristics and capacity to produce the thermostable direct hemolysine (TDH) of the Brazilian urease-positive strains. For comparison, we also included in the study 13 urease-negative cultures which have been isolated in the same laboratory.

For recovering *Vibrio*, fecal samples were enriched in alkaline peptone water (pH 8.5) supplemented with 2% NaCl and subcultured to thiosulfate-citrate-bile salts-sucrose agar (Difco Laboratories, Detroit, Mich.). Sucrose-negative (green) colonies were purified on blood agar, biochemically characterized as *V. parahaemolyticus* by using standard methods³, and tested for urease activity in Urea Broth (Difco Laboratories).

Determination of serovar was carried out by agglutination of the cultures, before and after boiling, on slides with 65 K and 11 0 antisera (Denka, Seiken Co., Tokyo, Japan). Production of TDH, the hemolysin responsible for the Kanagawa phenomenon (KP), was evaluated by two methods: beta hemolysis on Wagatsuma agar⁴ and a reversed passive latex agglutination quantitative test (Denka, Seiken Co.). This test was done by following the manufacturer directions.

From May 1989 to June 1990 we detected in the Laboratory Keizo Asami, in Recife, a total of 21 strains of *V. parahaemolyticus* from adult patients with gastroenteritis. All the patients positive

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for *V.parahaemolyticus*, presented with watery diarrhea without polymorphonuclear leukocytes in the stools. They acquired their infections locally after consumption of different kinds of seafood: oysters, shrimps, fishes, or octopus. Severity of illness could not be associated with biotype. That is, clinical manifestations were independent of whether the causal strain was ureolytic, produced TDH, or not. Human infections linked to KP-negative strains appears to be a commonplace, suggesting that in fact we do not yet know how *V.parahaemolyticus* causes gastrointestinal disease in humans².

Results of the serovar determinations, urease activity, and TDH production of the isolates are summarized in Table 1. Despite use of a large number of monospecific K antisera, serovar determination showed that five (24%) isolates were untypable, pointing out that many unrecognized serovars of *V.parahaemolyticus* still occur in tropical climates. The most prevalent serovars among the urease-negative cultures were 03:K58 (six, 46%) and 04:K4 (two, 15%). The serovar 04:K12, which predominates on the West-Coast of the United States^{1,10} was also found in Recife, but the Brazilian strain was urease negative and KP positive. Five different serovars were observed among the eight urease-positive *V.parahaemolyticus* strains, suggesting that such isolates were not acquired from a common source or derived from a single bacterial clone. The serovar 04:K53, KP-negative, was the most frequent (50%) among the urease-positive isolates. Interestingly, the serotype 05:K15, urease positive and KP-positive, isolated in Brazil Northeast some years ago⁶ was not identified in the present serie.

Most urease-positive strains produced less TDH than did the urease-negative ones or did not produce that hemolysin at all. On the other hand, only one (8%) of the urease-negative isolates was KP negative. Thus, in some strains, there was an apparent relationship between urease activity and an absence or diminution of TDH production. Such aberrant behaviour of our urease positive cultures was similar to that observed in British Columbia, where all isolates from diarrheal cases acquired locally were urease positive and KP negative⁸.

Whether that relationship was coincidental or resulted from an still unrecognized functional incompatibility, at the chromosomal level, between

the genes responsible for urease and TDH synthesis, remains to be determined. In contrast to ureolytic *Escherichia coli* strains¹³, the genes which govern urease activity in *V.parahaemolyticus* are not localized in plasmids¹⁸.

TABLE 1
Relationships among serovars, urease, and Kanagawa hemolysin production in 21 *V.parahaemolyticus* isolates

Serovars ^a	Nº of strains	Urease ^b	Homolysis on Wagatsuma agar	Hemolysin tiler ^d
01:K56	1	+	W ^c	8
03:K5	1	-	+	32
03:K58	6	-	+	16 to 128
03:KNT	1	+	-	0
04:K4	2	-	+	32
04:K10	1	-	+	64
04:K12	1	-	+	32
04:K53	4	+	-	0
04:KNT	1	+	+	4
05:KNT	1	-	+	32
010:KNT	1	+	W	2
010:KNT	1	-	-	0

a NT, Non typable

b Symbols: +, Positive at 24 h; -, Negative at 24h.

c W, Weakly positive

d determined by reversed passive latex agglutination.

RESUMO

Vibrio parahaemolyticus urease-positivos de pacientes diarréicos no Nordeste do Brasil.

De 21 linhagens de *Vibrio parahaemolyticus*, isoladas de fezes humanas, na costa Nordeste do Brasil, oito (38%) foram urease positivas. A maioria dessas linhagens, em contraste com as urease-negativas, não produziram a hemolisina responsável pelo fenômeno Kanagawa.

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