

VIBRIO METSCHNIKOVII AMONG DIARRHEAL PATIENTS DURING CHOLERA EPIDEMIC IN RECIFE BRAZIL

Vera MAGALHÃES (1), Adeíza BRANCO (2), Roberto de ANDRADE LIMA (3) & Marcelo MAGALHÃES (1)

SUMMARY

Although known since the last century, *Vibrio metschnikovii* was only appropriately described and recognized as a new species within the genus *Vibrio* in 1978. Rarely is the organism linked to human disease. Only once has *V. metschnikovii* been incriminated as responsible for human diarrhea, and affecting an old woman who suffered from diabetes and had a hepatoma. During the first two years of the present cholera epidemic, which reached Recife in March, 1992, we screened for vibrio nearly 4000 diarrheal fecal specimens submitted to a private clinical laboratory for detection of enteropathogenic microorganisms. Now, we report six cases of diarrhea associated with *V. metschnikovii* affecting individuals not suffering of any apparent underlying systemic illness.

KEYWORDS: *Vibrio metschnikovii*; Diarrhea; Vibrio; Cholera.

INTRODUCTION

Vibrio metschnikovii, an oxidase-negative and nitrate-negative species, described in 1888, redefined in 1978⁵, and extensively characterized in 1988 by FARMER et al.², is rarely isolated in human infections. Most nonhuman strains of this species were isolated from river water, oysters, lobsters and sewage⁷. Thus, *V. metschnikovii* is a vibrio species often isolated from the environment but rarely isolated from human clinical species. There are only four reports of human disease related or caused by *V. metschnikovii* and all have occurred in old or immunocompromised patients^{3,4}. In most of these human cases, *V. metschnikovii* caused bacteremia and was recuperated from blood cultures^{3,4}. To our knowledge, only once has *V. metschnikovii* been incriminated as responsible for human diarrhea; even so, the patient was an old person with diabetes and hepatoma⁸. It is rarely isolated from human feces and

is still a doubtful enteropathogen. Now, we report six cases of diarrhea associated with *V. metschnikovii* affecting individuals not suffering of any apparent systemic illness, during the cholera epidemic in Recife.

MATERIAL AND METHODS

During the first two years of the present cholera epidemic, which reached Recife on March, 1992, we screened for *Vibrio* all diarrheal fecal specimens submitted to a private clinical laboratory for detection of enteropathogenic microorganisms. To avoid biasing due to seasonality, we only considered the specimens received between March and December 1992 and the specimens received on equal period of 1993.

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 (TCBS; Difco Laboratories, De-

(1) Departamento de Medicina Tropical da Universidade Federal de Pernambuco (UFPE), Laboratório de Imunopatologia Keizo Asami (Lika-UFPE).

(2) Mestranda do Mestrado em Medicina Tropical da UFPE.

(3) Departamento de Medicina Clínica da UFPE.

Correspondence to: Vera Magalhães - Estrada do Arraial 2823/1701, 52051-380, Recife, PE, Brasil. Fone/Fax: (081) 4416169

troit, Mich). Colonies suspected of *Vibrio* were purified on sheep blood agar and biochemically characterized by using standard methods ¹.

RESULTS AND DISCUSSION

Nearly 4000 diarrheal fecal specimens were submitted to a private laboratory during the first two years of the present cholera epidemic. Between March and December, 1992, 2200 fecal samples were examined, and 1700 specimens were examined on equal period of 1993.

Interestingly, among 73 strains of non-cholera vibrios identified, 6 (8.2%) were classified as *V. metschnikovii* (table 1). Of these, five strains were recovered from diarrheal patients as the sole enteropathogen, and the last one jointly with *Salmonella* sp. All patients from whom *V. metschnikovii* was isolated were in good health until the development of the enteritis. Of the six patients, five were adults and three denied exposure to seafood. Clinically, the diarrhea was mild, brief, self-limited, and characterized by cramps and watery stools. No fecal leukocytes were seen on Gram stained smears.

The six *V. metschnikovii* strains showed a highly uniform biochemical testing profile. Positive tests for all the strains included growth on TCBS, growth at 40°C, requirement for NaCl, beta-hemolysis on sheep blood agar, sensitivity to 0/129 (150 µg per disk), hydrolysis of Tween 80 and esculin, arginine dihydrolase, and fermentation of sucrose, mannitol, inositol, and glycerol. Negative tests included oxidase, nitrate reduction, indole, O-nitrophenyl-B-D-galactopyranoside, growth in 8% NaCl, ornithine decarboxylase, gas production from glucose, and fermentation of arabinose, lactose, salicin, and sorbitol. Results of other tests, lysine decarboxylase and Voges-Proskauer, were strain dependent. Whereas the organism is easily separated

from other vibrios because it is oxidase-negative, such peculiarity impedes its rapid recognition as a member of the genus *Vibrio* in clinical laboratories. Perhaps, we detected somewhat many strains of *V. metschnikovii* because all yellow colonies seen on TCBS plates were submitted to biochemical identification simultaneously with oxidase testing, that is, oxidase-positivity was not a prerequisite for considering the strains as a vibrio.

The clinical significance of the present findings is not clear. We routinely use TCBS in our laboratory since 1989 and until the cholera epidemic started in 1992, no strain of *V. metschnikovii* had been recognized. In fact, fear of cholera changed the behavior of people now studied, individuals belonging to good or middle socioeconomic levels ⁷. The epidemic stimulated such people to submit for culturing many stool samples, yet from mild cases of diarrhea. People also altered their diet, abstaining from seafood and from visiting restaurants. Despite this, *V. cholerae* non-01 and *V. metschnikovii* were more frequently found on this year than on 1993, showing that seafood is not an usual source for human infections with these species. In contrast, on the second year of the epidemic, when the anxiety about cholera vanished and people returned to old alimentary habits, recovering of other vibrios increased steadily (p=0.002). This was mainly due to a rising in the frequency of *V. parahaemolyticus* and *V. furnissii*, the most common vibrios identified in Recife before the epidemic (Table 1). Strains of *V. cholerae* 01 also were more frequently found on the second year of the outbreak, an apparent paradox, since it would be expected a larger number of *V. cholerae* 01 on the first months of epidemic ⁷. Thus anxiety about cholera influences the isolation rate and the kind of vibrio species from people of good standard of life.

Albeit *V. metschnikovii* has at least a potential factor of virulence, a powerful cytotoxin with enterotoxic activity ⁸, further studies are necessary to decide whether present results are a laboratory curiosity, incidental to the cholera epidemic in Brazilian northeast, or whether they have some clinical relevance.

TABLE 1

Non-cholera *Vibrio* isolated during two years of the cholera epidemic in Recife, Brazil

Species	1992	1993
<i>V. cholerae</i> non-01	9	4
<i>V. alginolyticus</i>	1	1
<i>V. fluvialis</i>	3	3
<i>V. furnissii</i>	5	15
<i>V. metschnikovii</i>	5	1
<i>V. parahaemolyticus</i>	5	21
<i>Vibrio</i> spp.	1	1

RESUMO

Vibrio metschnikovii entre pacientes diarreicos durante epidemia de cólera no Recife, Brasil.

Embora reconhecido desde o século passado, o *Vibrio metschnikovii* só foi descrito e classificado de

forma apropriada dentro do gênero *Vibrio* em 1978. Raramente, essa bactéria tem sido relacionada com doença humana, acometendo habitualmente, pacientes imunodeprimidos. Há apenas um relato de gastroenterite a *V. metschnikovii*. Durante a recente epidemia de cólera, que acometeu o Recife em março de 1992, todos os espécimes fecais diarréicos submetidos a um laboratório particular de patologia clínica para diagnóstico microbiológico, foram também cultivados em meio ti-ossulfato, citrato, sais biliares e sacarose (TCBS). Analizou-se um total de 4000 amostras fecais durante os dois anos que se seguiram a chegada da cólera ao Recife. No presente, reportamos seis casos de diarreia a *V. metschnikovii* ocorridos nesse período.

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Recebido para publicação em 22/11/1995.

Aceito para publicação em 08/01/1996.