

Vibrio* spp. AND *Salmonella* spp., PRESENCE AND SUSCEPTIBILITY IN CRABS *Ucides cordatus

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SUMMARY

The presence of *Vibrio* spp. and *Salmonella* spp. in crabs marketed at the Bezerra de Menezes Ave., Fortaleza, State of Ceará, Brazil, was assessed between February and May, 2003. The number of individuals sampled in each one of the fifteen weekly samplings ranged between four and eight. Seven strains of *Salmonella*, from four different samplings, were identified, being five of them identified as serotype S. Senftenberg and two as S. Poona. All strains of *Salmonella* were sensitive to the tested anti-microbial drugs, with the exception of tetracycline and nalidixic acid, for which an intermediary sensibility was found. The MPN's for *Vibrio* ranged between 110/g and 110,000/g. Of the forty five *Vibrio* strains isolated from the crab samples, only 10 were identified up to the species level: two *V. alginolyticus* and eight *V. parahaemolyticus*. Bacteria belonging to the Enterobacteriaceae and Pseudomonaceae families were also identified, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pantoea agglomerans* and *Pseudomonas aeruginosa*. The proper cooking of the animals is recommended in order to avoid problems for the consumers of this crustacean.

KEYWORDS: Crabs; *Vibrio* spp.; *Salmonella* spp.; Susceptibility.

INTRODUCTION

Crabs are one of the most important natural resources in the estuarine regions of the Brazilian coast. They can be intensely exploited without reaching an overfishing threshold, mainly because the picking method allows the identification of the female individuals, different in size than the males, and their release back into the environment .

Every week, a great quantity of crabs are consumed in Ceará State, Brazil, most of which are hand-picked at the Parnaíba river delta¹⁷ and afterwards transported overland in trucks to the marketing places to be sold at Bezerra de Menezes Avenue, one of the most important selling points in Fortaleza city. The crabs are transported without any hygienic control, with the animals packed in fabric bags along with the mud residues of the mangroves they were taken from and, when they finally arrive, are left exposed for marketing, without any preventive hygienic treatment except for the washing done by the consumer himself.

Bacteria from genus *Salmonella* are the main etiologic agents of Food Transmitted Diseases (FTD) worldwide, being an important social and economic problem². Each year, from 800,000 to 4 million cases of infective diseases caused by non-typhoid *Salmonella* are estimated to occur in the United States. From those cases, about 500 deaths are caused by these infections and children are more frequently affected⁴. HOFER & REIS⁹ reported 25 events of food-related infections between 1982 and 1991 in the Brazilian southeastern and southern regions, each and

every one of them being caused by *Salmonella*, being *S. Typhimurium* the most common (52%).

Despite the fact that *Salmonella* is not normally isolated from fish and bivalves captured in the open sea, it can be found in marine products captured in contaminated waters.

Studies regarding the presence of *Vibrio parahaemolyticus*, a public health threat as the cause of gastroenteritic outbreaks in human populations, began after this bacterium was isolated from fish in several countries (United States, Brazil, Japan)^{1,12,26}. Therefore, the aim of this study was to assess the degree of *Salmonella* (presence or absence) and *Vibrio parahaemolyticus* contamination in the marketed crabs at Fortaleza, Ceará State, Brazil.

MATERIAL AND METHODS

Sampling: Fifteen weekly samplings were performed between February and May, 2003 (between four and eight live crabs (*Ucides cordatus*) each time, depending on their size). All of the 90 sampled animals were brought to the Laboratory of Fish and Environmental Microbiology of Instituto de Ciências do Mar (LABOMAR), Universidade Federal do Ceará, where they were killed and analyzed as planned.

Sample preparation: The crabs were scrubbed under a flux of tap

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water in order that any residues present on its carapace were removed. The claws and cephalothorax tissue were tested for the relevant bacteria. For the analysis of *Salmonella*, 25 g of tissue were immersed in 225 mL of lactose broth for 24 hours at 35° C. For *Vibrio parahaemolyticus*, 50 g were homogenized in 450 mL of a buffered phosphate saline solution (PBS). Dilutions of 10⁻¹ to 10⁻⁵ were done using 9 mL of the same saline solution used for the initial culture, with each inoculation done in triplicate, in 1% peptone water with 3% NaCl.

The tests used for the determination of *Salmonella* spp. and *Vibrio parahaemolyticus* were those of Methods for the Microbiological Examination of Foods⁶. The serology for *Salmonella* spp. was carried out at Enterobacteria Laboratory/Oswaldo Cruz Foundation, Rio de Janeiro.

Antigenic characterization: After their biochemical confirmation up to the sub-species level, the *Salmonella* spp. strains were submitted to a conclusive antigenic characterization, through a fast sero-agglutination technique¹⁸, using somatic and flagellar antisera produced by Enterobacteria Laboratory/FIOCRUZ.

Antimicrobial susceptibility test: The susceptibilities to antimicrobials were determined according to National Committee for Clinical Laboratory Standards¹⁵ recommendations using disks (OXOID) impregnated with Tetracycline (30 µg); Ampicillin (10 µg); Nalidixic acid (30 µg); Chloramphenicol (30 µg); Sulfamethoxazol-Trimethoprim (30 µg); Nitrofurantoin (300 µg); Cephalothin (30 µg); Cefoxitin (30 µg); Ceftriaxone (30 µg); Ceftazidime (30 µg); Gentamicin (10 µg); Streptomycin (30 µg); Ciprofloxacin (5 µg); Sulfonamide (23.75 µg); Enrofloxacin (5 µg); Imipenem (10 µg); and Aztreonam (5 µg) (Oxoid). For quality control, *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923 were tested under the same conditions and antimicrobial drugs.

RESULTS AND DISCUSSION

Seven out of 44 strains isolated from four different crab samples were confirmed to belong to genus *Salmonella* (15.2%). Our results, however, are in disagreement with those reported by REINHARD *et al.*¹⁹ who did not find any *Salmonella* strains in blue crabs (*Callinectes sapidus*) sampled at Chesapeake Bay, Virginia (USA).

Of the strains isolated from the contaminated samples, five were confirmed as *S. Senftenberg*, which is included among the five most commonly serovars isolated in Brazil in the last five years^{20,21}. Two strains were identified as *S. Poona*, with variable incidence in our environment, as it was reported by HOFER *et al.*¹⁰. These seven strains were isolated from four crab samplings, meaning that 26.6% of these crab samplings were contaminated with *Salmonella* spp.

In São Paulo, between January, 1996 and December, 2000, TAVECHIO *et al.*²⁴ isolated 4,581 strains of *Salmonella* from non-human source, including foodborne ones, identifying 123 different serotypes: *S. Enteritidis* (32.7%), *S. Senftenberg* (10.3%), *S. Hadar* (6.8%), *S. Agona* (5.1%), *S. Typhimurium* (2.4%) and others.

HATHA & LAKSHMANAPERUMALSAMY⁸ studied 730 fish and 276 crustaceans of the group Brachyura (crabs and swimming crabs) sampled at the fish markets of Coimbatore (Southern India), finding that 14.25% of the fish and 17.39% of the crustaceans were contaminated with *Salmonella* spp. The *S. Senftenberg* serotype was isolated from the crustaceans only, as it was the case of the present study.

Salmonella are widely disseminated microorganisms, able to easily spread to foods from one single contaminated source²². That is why the RDC 12 (National Sanitary Surveillance Agency-ANVISA)³ has a zero tolerance regarding this particular bacterium. Those microorganisms can be found in marine products captured in contaminated waters despite their not being usually isolated from fish and bivalves captured in the open sea. *Salmonella* spp. does not present a threat when present in raw products that will be cooked before consumption, since the cooking completely destroys it. Nonetheless, *Salmonella* can be transferred to other foods by cross-contamination.

The big concern regarding the presence of *Salmonella* in crabs is that, despite there would be none in the cooked animals, they can be reintroduced from the environment or the raw animals during their processing¹¹.

In the U.S.A., different non-typhoid *Salmonella* serovars have been associated to fish and crustaceans, and *S. Paratyphi* A, *S. Enteritidis* and *S. Typhi* has been the bacterium most associated to mollusc-related diseases⁷.

In a general way, it has been observed that the products not properly cooked or kept under deficient cooling conditions are those that present the greatest risks, with their excessive manipulation increasing such problem. *Salmonella*-related infections have the special feature that the presence of this organism does not alter the aspect, taste or other characteristics of a contaminated food¹³.

Besides *Salmonella*, other representatives genera of the Enterobacteriaceae and Pseudomonadaceae families were also isolated from the crab samples (Table 1). Some of them are soil-borne, while others have a fecal origin, showing the poor hygienic conditions of the marketed crabs.

The samples' MPN's for *Vibrio* ranged between 100/g and 1 10,000/g, with a high heterogeneity among the different samples. Forty-five strains

Table 1
Species identified in the 15 crab samples (90 animals) marketed at Fortaleza, State of Ceará, Brazil, in 2003

Family Vibrionaceae	Family Enterobacteriaceae	Family Pseudomonadaceae
<i>Vibrio parahaemolyticus</i>	<i>Salmonella</i> spp.	<i>Pseudomonas aeruginosa</i>
<i>Vibrio alginolyticus</i>	<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter cloacae</i> ,	
<i>Vibrio</i> spp.	<i>Pantoea agglomerans</i> , <i>Proteus mirabilis</i>	

of *Vibrio* were isolated, yet only 10 of them were identified to the species level, namely *V. parahaemolyticus* (eight) and *V. alginolyticus* (two).

Vibrio parahaemolyticus has been recognized as an important pathogen of humans and aquacultured animals especially in Asian countries. WONG *et al.*²⁸ examined 668 samples of seafood imported from Hong Kong, Indonesia, Thailand and Vietnam for *Vibrio parahemolyticus*, recovered strains of this bacterium from 315 (45.9%) samples. The incidence of *V. parahemolyticus* in products from Hong Kong was markedly higher than the incidence in products from the other countries. The incidence rates in shrimp, crab, snail, lobster, sand crab, fish and crawfish were 75.8, 73.3, 44.3, 44.1, 32.5, 29.3 and 21.1%, respectively. Likely, MATTÉ *et al.*¹⁴ working with pathogenic vibrios present in oysters from the southern coast of São Paulo State quantified *V. parahaemolyticus* that varied from < 3 to 1,200/100 g.

Between 1973 and 1998, forty gastroenteritis outbreaks were reported in the United States, 35% of which were caused by the consumption of raw oysters and, to lesser extent, of crabs, shrimps, lobsters and octopuses⁵.

THEOPHILO & VIEIRA²⁵, analyzing samples of raw and cooked crab meat sold at three beach restaurants in Praia do Futuro, Fortaleza, State of Ceará, Brazil, were able to confirm the presence of *V. parahaemolyticus* in 42.1% of the raw samples, and in 32.4% of the cooked ones.

Vibrio alginolyticus was initially classified as a *V. parahaemolyticus* biotype. They present a similar C-G percentage, yet having some phenotypic differences, with *V. alginolyticus* showing a saccharose fermentation capacity and growing at 10% NaCl. *V. alginolyticus* is autochthonous of the saline environment, and is usually detected in different ecological niches, being more abundant in the northern hemisphere, in the summertime, and a common inhabitant of tropical regions, where it is even part of the normal fish microbiota¹⁶. As a pathogen to humans, it is usually isolated from infections of cutaneous injuries, acquired in the marine environment.

Despite the high number of *Vibrio* species detected in the crab's raw meat, including those potentially harmful to humans, it is not possible to establish any correlation to acceptable levels because of the lack of regulatory limits for these organisms³ with the sole exception of *Vibrio parahaemolyticus*, and in this case only for foods intended to be consumed raw, which is not the case of crabs.

We can conclude that the isolation of different *Salmonella* serovars in the tested crabs is a matter of concern, it shows the influence of sewerage over the sites where the crabs are captured, being such a microorganism a natural inhabitant of the intestinal tract of both humans and animals. Its presence, therefore, indicates a direct or indirect fecal contamination, a situation that can be evidenced by the high frequency of *Escherichia coli* isolates.

As a cause of human morbidity, its frequency and/or persistence depend, essentially, on the involved serotype, the infecting dose, its ubiquitous characteristics, and the foods' hygienic and sanitary conditions. Regarding the hosts, some individuals are more susceptible to develop a septicemic condition, namely those with immune deficiencies or chronic infections, especially biliar or hepatic, some types of anemia, and HIV-positives²⁷.

Although all strains of *Salmonella* have been sensitive to the tested anti-microbial drugs, with the exception of tetracycline and nalidixic acid, for which an intermediary sensibility was found, such results must be assessed cautiously, since such organisms can easily acquire anti-microbial drug-resistant genes, an evolutionary mechanism to adapt themselves to their surrounding environment, which has serious repercussions over public health²³.

Since *Salmonella* was isolated from crabs, it is suggested that a Good Manufacturing Practices (GMP) program should be adopted during the cooking procedures. One should emphasize the importance to avoid live animals contact with the cooking recipients used to prevent cross-contaminations.

RESUMO

Vibrio spp. e *Salmonella* spp. em caranguejos, *Ucides cordatus*

Foram pesquisadas a presença de *Vibrio* spp. e de *Salmonella* spp. em caranguejos comercializados na Av. Bezerra de Menezes, Fortaleza, Ceará, Brasil, no período entre fevereiro e maio de 2003. O número de indivíduos em cada, das quinze coletas realizadas, semanalmente, variava entre quatro e oito dependendo do tamanho dos animais, totalizando um número de 90 (noventa) animais examinados. Foram identificadas sete cepas de *Salmonella* spp. provenientes de quatro coletas: cinco foram identificadas como sorovar *S. Senftenberg* e duas como *S. Poona*. Todas as cepas de *Salmonella*, isoladas das amostras de caranguejos, apresentaram sensibilidade aos antimicrobianos testados, com exceção de tetraciclina e ácido nalidíxico para os quais elas apresentaram uma sensibilidade intermediária. Os NMPs para *Vibrio* spp. variaram entre 110 e 110.000/g. Das quarenta e cinco cepas de *Vibrio* spp. isoladas das amostras de caranguejo, foram identificadas, até espécie, somente 10: duas de *V. alginolyticus* e oito de *V. parahaemolyticus*. Foram também identificadas bactérias pertencentes às famílias Enterobacteriaceae e Pseudomonadaceae: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pantoea agglomerans* e *Pseudomonas aeruginosa*. Recomenda-se que a cocção dos animais seja bem feita a fim de se evitar problemas para os consumidores dos crustáceos.

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