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CARACTERIZAÇÃO DAS ESPÉCIES DE VIBRIOS ISOLADAS EM AMOSTRAS DE ÁGUA DO MAR, PLÂNCTON E BIVALVES DA ZONA LITORÂNEA DO ESTADO DE SÃO PAULO

Tese (Doutorado) apresentada ao Programa de Pós-Graduação Interunidades em Biotecnologia USP/Instituto Butantan/IPT, para obtenção do Título de Doutor em Biotecnologia.

Área de Concentração: Biotecnologia

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São Paulo
2015

RESUMO

LAVEZZO, L. C. **Caracterização das espécies de vibrios isoladas em amostras de água do mar, plâncton e bivalves da zona litorânea do estado de São Paulo**. 2015. 104 f. Tese (Doutorado em Biotecnologia) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2015.

Os micro-organismos são essenciais para o meio ambiente e contribuem para a estabilidade dos ecossistemas. Sabe-se que as bactérias do gênero *Vibrio* são autóctones do ecossistema marinho, e, embora a diversidade e a patogenicidade estejam sendo descritas para diversas espécies, os problemas de saúde pública são devidos, geralmente, a *Vibrio cholerae*, *V. parahaemolyticus* e *V. vulnificus*. A proposta deste estudo foi caracterizar ao nível molecular as espécies de *Vibrio* isoladas de amostras de água do mar, plâncton e bivalves, coletadas no Canal de São Sebastião (n=78), Baixada Santista (n=37) e Ubatuba (n=17), analisar a susceptibilidade destes vibrios aos antibióticos e os principais genes associados à virulência (*ctxA, tcpA, stn/sto, tdh, trh*), além de relacionar os resultados obtidos com as diferenças espaciais e as atividades antropogênicas existentes nos locais estudados. Pelo antibiograma, foi possível observar sensibilidade à ciprofloxaxina (100%), ao meropenem (100%), ao ácido nalidíxico (99,2%), à tetraciclina (99,2%), e ao cloranfenicol (97,7%), resistência à ampicilina (62,4%) e à cefalotina (52,6%), além de uma alta porcentagem de múltipla resistência (64,7% em Ubatuba, 48,6% na Baixada Santista e 43% no Canal de São Sebastião) aos antimicrobianos. Os vibrios foram analisados quanto à presença de cinco genes associados à virulência e quatro (3,0%) isolados de água do mar da Baixada Santista foram positivos para o gene de virulência *stn/sto*, sendo três (PF620, PF623-1 e PF623-2) da espécie *V. parahaemolyticus* e um (PF482), *V. alginolyticus*. Pelos resultados obtidos por meio da Análise de Sequência Multilocus (MLSA), com os genes *pyrH*, *recA* e *rpoA,* foi possível identificar quatro espécies de vibrios em Ubatuba, um ambiente aquático com atividade antrópica mínima (*Vibrio alginolyticus*, *V. fluvialis*, *V. campbellii* e *V. harveyi)*; onze espécies de vibrios no Canal de São Sebastião, área com moderado impacto humano (*V. fluvialis*, *V. alginolyticus, V. campbellii, V. rotiferianus*, *V. harveyi, V. diabolicus*, *V. atypicus*, *V. coralliilyticus*, *V. maritimus*, *V. parahaemolyticus* e *V. tubiashii*); e, onze espécies na Baixada Santista, área com alta atividade antrópica (*V. alginolyticus, V. parahaemolyticus*, *V. rotiferianus*, *V. campbellii, V. harveyi, V. communis*, *V. maritimus*, *V. fluvialis*, *V. fortis*, *V. natriegens* e *V. navarrensis*).

Palavras-chave: *Vibrio*. Ecossistemas marinhos. Estado de São Paulo. Antibiograma. Genes associados à virulência. MLSA.

ABSTRACT

LAVEZZO, L. C. **Characterization of vibrio species isolated from seawater, plankton and bivalves samples from the São Paulo State coastal zone**. 2015. 104 p. Ph. D. thesis (Biotecnology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2015.

Microorganisms are vital to the environment and play an important role in the stability of ecosystems. Bacteria of the genus *Vibrio* are autochthones of marine ecosystems and, although the diversity and pathogenicity have been described for several species, public health problems are generally due to *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus.* The purpose of this study was to characterize at the molecular level *Vibrio* species isolated from seawater, plankton and bivalves samples collected from Canal de São Sebastião (n=78), Baixada Santista (n=37) and Ubatuba (n=17), to analyze their antimicrobial susceptibility, the major virulence-associated genes (*ctxA, tcpA, stn/sto, tdh, trh*) and to bring these results into relation with the different levels of anthropogenic activity of the studied sites. The antimicrobial susceptibility tests showed sensitivity to ciprofloxacin (100%), meropenem (100%), nalidixic acid (99.2%), tetracycline (99.2%), chloramphenicol (97.7%), resistance to ampicillin (62.4%) and cephalothin (52.6%), and a significant percentage of multidrug resistance (64.7% in Ubatuba, 48.6% in Baixada Santista, and 43% in Canal de São Sebastião). Five virulence-associated genes were examined and four (3.0%) seawater isolates collected from Baixada Santista proved positive for *stn/sto*, three of them were identified as *V. parahaemolyticus* (PF620, PF623-1 and PF623-2) and one, as *V. alginolyticus* (PF482). MLSA allowed the identification of four *Vibrio* species from Ubatuba, an environment of the coastal region with low anthropogenic activities: *V. alginolyticus* (n=7), *V. fluvialis* (n=5), *V. campbellii* (n=3), and *V. harveyi* (n=2). In Canal de São Sebastião, an area with medium anthropogenic activities, eleven species were identified: *V. fluvialis* (n=30), *V. alginolyticus* (n=24), *V. campbellii* (n=8), *V. rotiferianus* (n=5), *V. harveyi* (n=4), *V. diabolicus* (n=2), *V. atypicus* (n=1), *V .coralliilyticus* (n=1), *V. maritimus* (n=1), *V. parahaemolyticus* (n=1), and *V. tubiashii* (n=1). In Baixada Santista, an area with high anthropogenic activities, the eleven identified species were: *V. alginolyticus* (n=11), *V. parahaemolyticus* (n=8), *V. rotiferianus* (n=4), *V. campbellii* (n=3), *V. harveyi* (n=3), *V. communis* (n=2), *V. maritimus* (n=2), *V. fluvialis* (n=1), *V. fortis* (n=1), *V. natriegens* (n=1), and *V. navarrensis* (n=1).

Keywords: *Vibrio*. Marine ecosystems. State of São Paulo. Antibiogram. Virulence-associated genes. MLSA.

1 INTRODUÇÃO

Os micro-organismos são essenciais para o meio ambiente, contribuem para a estabilidade dos ecossistemas, fazem a ciclagem de compostos químicos, incluindo a degradação de poluentes (STALEY, 1998). Dentre os micro-organismos marinhos, as bactérias do gênero *Vibrio* fazem parte da microbiota autóctone do ecossistema marinho e estuarino, permanecendo tanto no sedimento como na coluna d'água, sendo comumente isolados de peixes e crustáceos, ou associados com plantas, vivendo como simbiontes ou patógenos (DUMONTET et al., 2000).

Essas bactérias são capazes de degradar hidrocarbonetos aromáticos policíclicos (RAMAIAH et al., 2000) e têm um papel importante na reciclagem de quitina no ambiente aquático (HUQ; COLWELL, 1994). Algumas espécies são patogênicas, capazes de causar lesões em organismos marinhos e infecções e gastroenterites em humanos através da exposição de feridas em água ou pelo consumo de água ou frutos do mar crus ou mal cozidos, sendo que as principais espécies diretamente implicadas em surtos de veiculação hídrica e alimentar, segundo a Organização Internacional de Alimentos e Agricultura das Nações Unidas (FAO) são *V. cholerae*, *V. parahaemolyticus* e *V. vulnificus*.

Diante da importância das bactérias no ambiente marinho, o grupo de pesquisa do Laboratório de Ecologia Microbiana Molecular do Instituto de Ciências Biomédicas da Universidade de São Paulo, liderado pela Professora Dra Irma Nelly G. Rivera, vem desenvolvendo trabalhos com vibrios, desde 2005, na região costeira do Estado de São Paulo.

No âmbito desta linha de pesquisa, o presente estudo procurou caracterizar as espécies de vibrios isoladas de amostras de água do mar, plâncton e bivalves da Baixada Santista, Canal de São Sebastião e Ubatuba, ecossistemas com diferentes níveis de impacto antropogênico. O conhecimento da dinâmica e diversidade de vibrios deverá trazer benefícios econômicos e estratégicos relacionados com a identificação de micro-organismos potencialmente exploráveis em processos biotecnológicos, como na decomposição de quitina e degradação de hidrocarbonetos aromáticos (COLWELL, 1997; SOUZA-SALES, 2009), além do prognóstico e prevenção de doenças emergentes em seres humanos e em animais, em vigilância epidemiológica e em estudos de microbiologia alimentar.

7 CONSIDERAÇÕES FINAIS

- Neste estudo, os isolados de *Vibrio* spp. apresentaram sensibilidade ao ácido nalidíxico, à ciprofloxaxina, ao cloranfenicol, ao meropenem e à tetraciclina e resistência à ampicilina e à cefalotina, além de uma alta porcentagem de múltipla resistência aos antimicrobianos: 64,7% em Ubatuba, 48,6% na Baixada Santista e 43% no Canal de São Sebastião.

- Em nossos isolados não foram encontrados vibrios potencialmente patogênicos.

- Há uma reduzida base de dados para o gene *rpoD*, o que dificultou as análises dos isolados com as sequências de referências.

- Utilizando os genes *housekeeping* nas análises filogenéticas, os isolados foram identificados ao nível de espécie, apresentando, em alguns casos, divergências nas espécies membros do "*Vibrio core group"*.

- Pelos resultados obtidos com MLSA, utilizando os genes *pyrH*, *recA* e *rpoA,* foi possível identificar quatro espécies de vibrios em Ubatuba, um ambiente aquático com atividade antrópica mínima (*Vibrio alginolyticus*, *V. fluvialis*, *V. campbellii* e *V. harveyi)*; onze espécies de vibrios no Canal de São Sebastião, área com moderado impacto humano (*Vibrio fluvialis*, *V. alginolyticus, V. campbellii, V. rotiferianus*, *V. harveyi, V. diabolicus*, *V. atypicus*, *V. coralliilyticus*, *V. maritimus*, *V. parahaemolyticus* e *V. tubiashii*); e, onze espécies na Baixada Santista, área com alta atividade antrópica (*Vibrio alginolyticus, V. parahaemolyticus*, *V. rotiferianus*, *V. campbellii, V. harveyi, V. communis*, *V. maritimus*, *V. fluvialis*, *V. fortis*, *V. natriegens* e *V. navarrensis*).

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