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PRESENCE OF *BLA*$_{\text{TEM-116}}$ GENE IN ENVIRONMENTAL ISOLATES OF *AEROMONAS HYDROPHILA* AND *AEROMONAS JANDAEI* FROM BRAZIL

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ABSTRACT

It is known that *Aeromonas* spp. possess different chromosomal β-lactamase genes. Presence and phenotypic expression of *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{CTX-M}}$ ESBL-encoding genes were investigated in environmental water isolates of *Aeromonas hydrophila* and *Aeromonas jandaei*. Presence of *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M}}$ genes was not observed, and *bla*$_{\text{TEM}}$ gene was verified in 91% of the isolates. Sequencing of 10 fragments showed the occurrence of *bla*$_{\text{TEM-116}}$.

Key words: *Aeromonas, bla*$_{\text{TEM-116}}$, environmental isolates.

Members of the genus *Aeromonas* have been associated with a wide range of illnesses in humans, including gastrointestinal disorders and systemic infections in both immunocompromised and healthy hosts (4). Several studies have shown the presence of *Aeromonas* spp. in food and drinking water samples, suggesting that these sources may act as dissemination vehicles of the human pathogen, with implications in the public health. Furthermore, it is known that *Aeromonas* spp. are among the few microorganisms harboring different chromosomal β-lactamase genes, including *cphA* (also named *imiH*), *cepH* and *ampH*, encoding class B, C and D β-lactamases, respectively (1).

Antibiotic resistance has been classified by the World Health Organization as one of the three major public health threats of the 21st century (6). The rapid emergence of antibiotic resistance among bacteria is, to a great extent, due to the dissemination of antibiotic resistance genes by horizontal transfer mediated by plasmids, transposons and integrons (5). Among the clinical populations of Gram-negative microorganisms, *bla*$_{\text{TEM-1}}$ is the most frequently detected antimicrobial resistance gene and, although its expression results in penicillin resistance, diverse point mutations in the *bla*$_{\text{TEM-1}}$ gene have contributed to the emergence of TEM-type extended-spectrum β-lactamases (ESBLs), resulting in simultaneous resistance to penicillins and broad-spectrum cephalosporins (8). Although, almost all previous studies and efforts to control the dissemination of these genes have been based on isolates from clinical samples, antibiotic resistance genes can also occur in nonpathogenic bacteria, which can then be transferred via lateral gene transfer (6).

The aim of this study was to investigate the presence and phenotypic expression of *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{CTX-M}}$ ESBL-

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encoding genes in 87 environmental water isolates of *Aeromonas hydrophila* (n=41) and *Aeromonas jandaei* (n=46). The identification of *Aeromonas* species was performed as previously described (1). All isolates were screened for ESBL production by a double-disc synergy test using clavulanic acid (amoxicillin-clavulanate disk, 20/10 µg) and cloxacillin (200 µg/ml-containing Mueller-Hinton agar plates) as ESBL and AmpC inhibitors, respectively, and ceftazidime, cefpodoxime and cefotaxime-containing disks as ESBL substrates (9). Presence of ESBL-encoding genes was evaluated by PCR (2), and plasmid extraction was carried out using the commercial kit Wizard Plus SV Miniprep (Promega-USA). Also the search for class 1 integron was carried out according to previous work (7).

Although production of ESBL was not confirmed by phenotypic methods, and no amplification of the bla_{SHV} and bla_{CTX-M} genes was observed, the presence of the bla_{TEM} gene was verified in 97.6% (40/41) and 85% (39/46) of *A. hydrophila* and *A. jandaei* isolates, respectively. Nucleotide sequencing showed 100% sequence identity with the $bla_{TEM-116}$ gene (GenBank accession numbers FJ767900 to FJ767909). Plasmids were found in 24.4% (10/41) of *A. hydrophila* and in 34.9% (16/46) of *A. jandaei* isolates, suggesting no association between plasmid occurrence and presence of bla_{TEM} genes. Also the association of bla_{TEM} genes with the occurrence of class 1 integrons was not observed.

The present work raises a question concerning the possible origin of bla_{TEM} genes and their dissemination among environmental isolates. According to the data shown herein, the presence of these genes could not be associated to the occurrence of plasmids, suggesting a chromosomal location in *A. hydrophila* and *A. jandaei* isolates in Brazil. On the other hand, the $bla_{TEM-116}$ gene variant is closely related to the bla_{TEM-1} gene, which does not possess ESBL activity. In this work, the *Aeromonas* species harboring the $bla_{TEM-116}$ gene did not showed ESBL activity. In fact, this absence of ESBL activity was also observed in a $bla_{TEM-116}$ gene-carrying *Klebsiella pneumoniae* strain isolated in a teaching hospital in São Paulo, Brazil (3).

In conclusion, these data suggest that presence of bla_{TEM}-like genes in *Aeromonas* species recovered from natural water reservoirs could be intrinsic. Thus, risk of waterborne diseases owing to domestic and industrial uses of freshwater should be re-examined from the increase of bacterial resistance point of view. Finally, additional investigation about plasmidial or chromosomal occurrence of $bla_{TEM-116}$ in environmental isolates of *Aeromonas* is worthy of evaluation.

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REFERENCES

1. Balsalobre, L.C.; Dropa, M.; Lincopan, N.; Mamizuka, E.M.; Matté, G.R.; Matté, M.H. (2009). Detection of metallo-beta-lactamases-encoding genes in environmental isolates of *Aeromonas hydrophila* and *Aeromonas jandaei*. *Lett. Appl. Microbiol.* [In press].
2. Cao, V.; Lambert, T.; Nhu, D.Q.; Loan, H.K.; Hoang, N.K.; Arlet, G.; Courvalin, P. (2002). Distribution of extended-spectrum beta-lactamases in clinical isolates of *Enterobacteriaceae* in Vietnam. *Antimicrob. Agents Chemoter*. 46: 3739-3743.
3. Dropa, M.; Balsalobre, L.C.; Lincopan, N.; Mamizuka, E.M.; Cassetari, V.C.; Matté, G.R.; Matté, M.H. Emergence of Klebsiella pneumoniae carrying the novel extended-spectrum β-lactamase gene variants bla_{SHV-40}, $bla_{TEM-116}$ and the class 1 integron-associated bla_{GES-7}, Brazil. *Clin. Microbiol. Infect.* [In press]
4. Henriques, I.; Moura, A.; Alves, A.; Saavedra, M.J.; Correia, A. (2006). Analysing diversity among beta-lactamase encoding genes in aquatic environments. *FEMS Microbiol. Ecol.* 56(3):418-29.
5. Lachmayr, K.L.; Kerkhof, L.J.; Dirienzo, A.G.; Cavanaugh, C.M.; Ford, T.E. (2009). Quantifying nonspecific TEM beta-lactamase (bla_{TEM}) genes in a wastewater stream. *Appl. Environ. Microbiol.* 75(1):203-11.
6. Lévesque, C.; Piché, L.; Larose, C.; Roy, P.H. (1995). PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob. Agents Chemother*. 39(1): 185-191.
7. Mroczkowska, J.E.; Barlow, M. (2008) Recombination and selection can remove bla_{TEM} alleles from bacterial populations. *Antimicrob. Agents Chemother*. 52(9):3408-10.
8. Weldhagen, G.F.; Poirel, L.; Nordmann, P. (2003). Ambler class A extended-spectrum beta-lactamases in Pseudomonas aeruginosa: novel developments and clinical impact. *Antimicrob. Agents Chemother*. 47(8):2385-92.