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Letters to the Editor

Reservoir of Antimicrobial Resistance Determinants Associated with Horizontal Gene Transfer in Clinical Isolates of the Genus *Shewanella*▽

Although *Shewanella* is usually considered an environmental genus, different clinical infections have appeared in recent years (4, 7). Treatment of such infections is difficult due to the lack of knowledge concerning the natural antimicrobial resistance as well as the recommended antibiotic treatment of their infections (11). The aim of our study was to investigate the antimicrobial resistance mechanisms acquired by this genus in the nosocomial environment.

All isolates identified as *Shewanella* spp. ($n = 10$) by the use of standard biochemical tests were collected in a public hospital of Argentina during 2005 and 2006. In Argentina, the relative frequency of isolates positively identified as harboring *Shewanella* spp. among infections by nonfermentative Gram-negative bacilli (NFGNB), excluding the important pathogens *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, is only 2.5% of the total number of isolates. Amplification and sequencing of the 16S rRNA gene was used to identify the isolates (4, 12). Three isolates were identified as *Shewanella putrefaciens* and seven as *Shewanella algae* (Table 1).

PCR amplifications using total DNA were performed according to the instructions of the manufacturer (Promega) by the use of specific primers for evaluation of the presence of antimicrobial resistance determinants associated with horizontal gene transfer (most of them usually found in our Gram-negative bacterial isolates): (i) integron integrase genes (*intI1*, *intI2*, and *intI3*) (6, 10); (ii) 13 β-lactamase-like genes, namely, the bla_{OXA-58}, bla_{OXA-48}, bla_{TEM-1}, $bla_{CTX-M-2}$, $bla_{SHV-like}$, bla_{SCO-1}, bla_{PER-2}, $bla_{GES-like}$, $bla_{VEB-like}$, $bla_{IMP-like}$, $bla_{VIM-like}$, bla_{SPM-1}, and bla_{OXA-23} genes (5, 6, 9); and (iii) *sul1*, *sul2*, and *sul3* genes (1).

We found the type 1 integrase gene in 9 isolates, while the type 2 integrase gene was found in 6 isolates (Table 1). In order to identify the inserted gene cassettes within the variable region of integrons, PCR cartography and sequencing were performed as previously described (6, 10).

Concerning the variable region of class 1 integrons (vr-1), we found the presence of the *dfrA1* gene cassette in isolates Sa9, Sa82, and Sa392 and the *dfrA1-aadA1* array in isolates Sa74, Sa10, and Sa2 (Table 1). We were unable to identify gene cassettes within the vr-1 for three isolates (Sp117, Sp95, and Sp31). Concerning class 2 integrons, only the variable region of Sa2 harboring *dfrA1-sat2-aadA1*-orfX-*ybfA-ybfB-ybgA*, Sa9 harboring *dfrA1-sat2*, and Sa10 harboring the *dfrA1* gene cassette could be identified (Table 1).

When we investigated the presence of 13 β-lactamase genes, positive amplification was obtained for the 3 isolates of *S. putrefaciens* (Table 1) by the use of primers for the amplification of the carbapenemase bla_{OXA-48} gene previously found harbored in a transposon of a *Klebsiella pneumoniae* isolate from France (9). Sequence analysis revealed 99% identity over a 690-bp length with this gene, 83% identity with the sequence of bla_{OXA-54} previously described as present in *S. oneidensis* and suggested as the progenitor of the bla_{OXA-48} (8), and 78% identity with the sequence of a chromosome-borne bla_{OXA} gene that we have identified in *S. putrefaciens* CN-32 (CP000681.1) by the use of BLAST software (version 2.0). The meropenem (MEM) and imipenem (IMP) MICs were determined according to CLSI guidelines (3). As was shown in Table 1, no clear contribution of this gene to carbapenem resistances could be established.

Since there is no information about the occurrence of plasmids in *Shewanella* spp., we performed plasmid extraction using a QIAprep Spin Miniprep kit (Qiagen) to see whether our isolates contained plasmids. We found that plasmids were present in 2 out of 10 isolates (Sa2 and Sp95). We performed PCRs for commonly incompatible groups (IncP, IncW, and IncA/C) found in our bacterial population (reference 2 and data not shown) as well as plasmid extraction followed by *Escherichia coli* transformation and selection with the corresponding antibiotics, but all experiments gave negative results.

TABLE 1. Antimicrobial resistance mechanisms found among *Shewanella* clinical isolates[a]

Isolate[b]	Yr	Source	*intI1*[c]	*intI2*	Variable region of class 1 integrons	Variable region of class 2 integrons	*sul1*	*sul2*	*sul3*	bla_{OXA-48}-like gene(s)	MIC[d] (μg/ml)	
											MEM	IPM
Sp117	2005	Otic secretion	+	−	NGC[e]		+	+	−	+	4	1
Sp95	2005	Ocular secretion	+	−	NGC		+	+	−	+	0.25	0.5
Sp31	2005	Soft tissue	+	−	NGC		−	−	−	+	0.125	0.25
Sa9	2006	Blood culture	+	+	*dfrA1*	*dfrA1-sat2*	+	+	−	−	0.125	2
Sa78	2005	Soft tissue	−	−				+	−	−	0.25	4
Sa74	2006	Otic secretion	+	+	*dfrA1-aadA1*	NGC[e]	+	+	−	−	0.0094	4
Sa10	2006	Leg ulcer	+	+	*dfrA1-aadA1*	*dfrA1*	+	+	−	−	1	8
Sa82	2006	Soft tissue	+	+	*dfrA1*	NGC	+	+	−		0.38	0.25
Sa392	2006	Leg ulcer	+	+	*dfrA1*	NGC	+	+	−	−	1	4
Sa2	2006	Otic secretion	+	+	*dfrA1-aadA1*	*dfrA1-sat2-aadA1-orfX*	+	−	−	−	0.032	0.25

[a] The gene cassette arrays in the variable region of class 1 and 2 integrons, the presence of *sul* genes, the presence of bla_{OXA-48}-like genes, and the meropenem and imipenem MICs, as well as the sources of the samples, are shown.
[b] Isolates of the study: Sp for *Shewanella putrefaciens* ($n = 3$) and Sa for *Shewanella algae* ($n = 7$).
[c] − and +, negative and positive, respectively, for the presence of *intI1*, *intI2*, *sul1*, *sul2*, *sul3*, or bla_{OXA-48}-like genes by PCR with specific primers.
[d] Determinations of meropenem (MEM) and imipenem (IPM) MICs were performed according to CLSI recommendations (3).
[e] NGC, negative for the amplification of gene cassettes.

We found not only high levels of dispersion of genetic elements usually associated with horizontal gene transfer for both species but also distinctive epidemiology characteristics, since *S. putrefasciens* isolates possess the bla_{OXA-48} gene and class 1 integrons, while *S. algae* isolates possess class 1 and 2 integrons with different arrays of cassettes in the two species (Table 1).

Given that *Shewanella* is well known as an environmental genus and that almost all isolates from our study harbored integrons and other relevant determinants of resistance (such as the carbapenemase bla_{OXA-48}) usually associated with horizontal gene transfer, species of this genus could be considered to represent not only a potential reservoir but also a vector of antimicrobial resistance mechanisms in hospital settings and the environment, with transmission occurring in both directions.

Nucleotide sequence accession number. The partial sequence of the bla_{OXA-48} gene has been submitted to GenBank and assigned accession no. HM755942.

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