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Antibiotic resistance of *Aeromonas hydrophila* strains isolated from several sources

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Aeromonas hydrophila, a motile gram-negative rod, causes several diseases among poecilotherm and homeotherm animals, including haemorrhagic septicemia, red sore, gastroenteritis and endocarditis [2,3,5]. Several bacterial phenotypic properties, such as resistance to antimicrobial drugs or virulence determinants have been demonstrated to be plasmid encoded. The presence of plasmids in these pathogenic microorganisms may possess a potential public health hazard, since they may be transferred from animals to humans either directly or indirectly [1,4,6,7].

In this study the antibiotic resistance profiles of *A. hydrophila* strains isolated from water environments and animals (shellfish and fish) were analyzed. Furthermore, the loss of the resistance to any antimicrobial agents after curing experiments of the strains was also considered. Drug sensitivity patterns of 60 strains of *A. hydrophila* isolated from animals (shellfish and fish) and water (freshwater and seawater) samples were determined by disk diffusion using Mueller-Hinton agar (BioMerieux, Spain). The following chemotherapeutic agents and concentrations were used (µg/ disk): amikacin (30), ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (30), colistin (10), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), nitrofurantoin (300), B polimyxin (300 U), prystanamycin (15), streptomycin (10), sulphadiazine (1000), sulphamethoxazole-trimethoprim (1.25 + 23.75), tetracycline (30), and tobramycin (10). All the antibiotic disks were supplied by BioMerieux.

Curing experiments were carried out using acridine orange (Sigma, USA), following a modification of the techniques described by Winckler et al. [8]. Cells were grown in Brain-Heart-Infusion broth (BHIB, Difco, USA) for 24 h, and 2-ml aliquots were added to 1-ml of fresh broth, incubating for 3 h at 26°C. Then, 1-ml of a solution of acridine orange (20 µg/ml) was added, and the culture was centrifuged at 3,500 x g for 20 min. The supernatant was eliminated, and 2-ml of fresh BHIB were added to the pellet, incubating at 35°C for 2-6 h. After this period of time the strains were tested in relation to the loss of antibiotic resistance and plasmid profiles.

The overall percentage of drug-resistance (Table 1) indicated that more than 90% of the strains were resistant to ampicillin (91.7%), cephalothin (91.7%), tetracycline (96.7%), and prystanamycin (93.3%), which may be considered like a natural or chromosomal resistance to these drugs. On the other hand, percentages of resistance lesser than 5% were obtained for gentamicin (3.3%) and amykacin (0%).

Although the percentage of resistance of all three groups of strains was quite similar, some differences were found according to the source of isolation (Table 1). In fact, resistance to gentamicin was only detected on the strains from seawater. Similarly, higher percentages of resistance to carbenicillin, chloramphenicol, sulphamethoxazole-trimethoprim, and nalidixic acid were obtained on the strains isolated from water in comparison with those from strains of animal origin. For streptomycin, tobramycin, and kanamycin the percentages of resistance obtained for the freshwater strains were significantly lower ($p < 0.01$) than for the other strains. Finally, neomycin resistance was more frequently detected among the strains isolated from marine animals (20%) than from the other environments (about 4%).

All the derivative isolates from the acridine orange treatment were tested for plasmid content and drug resistance patterns. Table 1 reports the percentages of drug-resistance presented by the strains after the curing assay. The strains of *A. hydrophila* isolated from the three environments carried resistance genes located in the bacterial chromosome for the antibiotics ampicillin, cephalothin, tetracycline, carbenicillin, prystanamycin and nitrofurantoin. In contrast, the cured plasmidless strains lost simultaneously their resistance to tobramycin, neomycin, gentamicin, sulphadiazine and kanamycin. The resistance to nalidixic acid, streptomycin, sulphamethoxazole-trimethoprim, chloramphenicol and colystin are linked to chromosomal and plasmid genes.

Table 1. Resistance to 18 chemotherapeutic agents in the strains of *A. hydrophila* depending on the source of isolation before and after curing experiments

Drugs	Source							
	Freshwater (n=23)		Seawater (n=27)		Animals (n=10)		Total (n=60)	
	Before[a]	After[a]	Before	After	Before	After	Before	After
Ampicillin (Am)	87[b]	87	92.6	92.6	100	100	91.7	91.7
Amikacin (AN)	0	0	0	0	0	0	0	0
Carbenicillin (Cb)	69.6	69.6	74.1	74.1	10	10	61.7	61.7
Cephalothin (Cf)	87	87	92.6	92.6	100	100	91.7	91.7
Chloramphenicol (C)	65.2	43.5	51.9	48.1	20	20	51.7	41.7
Colistin (Cl)	26.1	21.7	18.2	11.1	20	10	21.7	15
Gentamicin (Gm)	0	0	7.4	0	0	0	3.3	0
Kanamycin (K)	4.3	0	18.5	0	10	0	11.7	0
Nalidixic acid (NA)	60.9	34.8	59.3	44.4	10	10	51.7	35
Neomycin (N)	4.3	0	3.7	0	20	0	6.7	0
Nitrofurantoin (FM)	78.3	78.3	81.5	81.5	60	60	76.7	76.7
Polimyxin B[c] (PB)	4.3	ND	11.1	ND	10	ND	8.3	ND
Prystanamycin (Pr)	87	87	96.3	96.3	100	100	93.3	93.3
Streptomycin (S)	43.5	13	85.2	33.3	80	20	68.3	23.3
Sulphadiazine (Sd)	78.3	0	74.1	0	60	0	73.3	0
Sulphamethoxazole (SXT)	69.6	13	63	22.2	30	10	60	16.7
Tetracycline (Te)	95.6	95.6	96.3	96.3	100	100	96.7	96.7
Tobramycin (NN)	8.7	0	14.8	0	20	0	15	0

[a] Before and after curing experiments

[b] Percentage of resistant strains.

[c] All the strains resistant to Polimyxin B did not harbored plasmid
ND: Not determined

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