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Antimicrobial Susceptibilities and Detection of Transferable R-Plasmids from *Aeromonas hydrophila* in Thailand

K. SAITANU

Department of Veterinary Public Health
Faculty of Veterinary Science
Chulalongkorn University
Bangkok 10330, Thailand

A. CHONGTHALEONG

Department of Microbiology
Faculty of Medicine
Chulalongkorn University
Bangkok, Thailand

M. ENDO, T. UMEDA, K. TAKAMI, T. AOKI and T. KITAO

Department of Fisheries
Faculty of Agriculture
Miyazaki University
Miyazaki, Japan

Abstract

Sixty eight strains of *Aeromonas hydrophila* isolated in Thailand from cultured fishes, hu-mans, water of fishponds, and soft-shell turtles were tested for their susceptibility to 19 antimicro- bial agents. All strains had intrinsic resistance to ampicillin. Fourteen strains were found to be susceptible to all drugs used except ampicillin, while the remaining 54 strains were resistant to either cephazoline, chloramphenicol, streptomycin, tetracycline, sulfamonomethoxine, trimethoprim or furazolidone. The antibacterial activity of the pyridone carboxylic acids (new quinolones) was highest against all strains. Strains carrying plasmids encoded with resistance to tetracycline were detected in 9 of 54 resistant strains all of which were isolated from cultured snakehead *(Ophicephalus striatus)*. The R-plasmids encoding resistance to chloramphenicol, tetra-cycline and sulfamonomethoxine were predominant. One strain encoded only for resistance to tetracycline.

Introduction

Infections with *Aeromonas hydrophila* have occurred frequently in clariid catfish *(Clarias batrachus)* culture ponds in Thailand (Saitanu 1986). Occasion-

ally, *A. hydrophila* strains have also been isolated from cases of human septicemia and from feces in human diarrhea (Lelaratsmi and Aswapokee 1979; Thamlikikul and Danchaivijitr 1981; Reinprayoon et al. 1985) Chemotherapeutic agents have been used to treat infections with *A. hydrophila* in cultured fish as well as in humans (Lelaratsmi et al. 1979; Saitanu and Chalarak 1983). The incidence of drug resistant strains of *A. hydrophila* has increased in fish farms in Thailand as a result of the wide use of chemotherapeutics (Navarat et al. 1979; Saitanu and Wongsawang 1982; Reungprach and Kesomchandra 1983; Saitanu and Chalarak 1983).

In 1983, there were massive losses of freshwater fish in Thailand. Most of the moribund fishes had severe ulcers on all parts of the body and *A. hydrophila* usually could be isolated from these lesions. (Boonyaratpalin et al. 1983). During the epizootic, several antimicrobial agents were used in an attempt to control the disease (Saitanu and Poonsuk 1984). This widespread use of antimicrobial agents created concern about the development of drug resistance.

This paper describes the drug susceptibility and the presence of transferable R-plasmids of *A. hydrophila* isolates from Thailand.

Materials and Methods

Bacterial Strains and Culture Conditions

Sixty-eight strains of *A. hydrophila*, collected between 1977 and 1988, were identified according to Bergey's Manual (Popoff 1984). The sources of the cultures are shown in Table 1. All strains were lyophilized in skimmed milk and stored in the refrigerator at 10°C. In this study, the organisms were inoculated in nutrient broth, subcultured on nutrient agar, and incubated at 30°C.

Table 1. The sources of 68 strains of *Aeromonas hydrophila* from Thailand.

Sources	No.	Year of isolation
Human	20	1983-1988
Snakehead *(Ophicephalus striatus)*	24	1983
Walking catfish *(Clarias batrachus)*	7	1977-1983
Carp *(Cyprinus carpio)*	1	1983
Striped catfish *(Pangasius pangasius)*	1	1983
Sand-goby *(Oxyeleotris marmoratus)*	1	1983
Bluespot grey mullet *(Valamugil siheli)*	1	1983
Soft-shell turtle *(Trionyx cartilagenous)*	2	1983
Water and sediment from cultured fishponds	11	1983

Antimicrobial Susceptibility Test

Minimal inhibitory concentrations (MIC) of various antimicrobial agents against strains of *A. hydrophila* were determined by serial two-fold agar dilution method (Aoki et al. 1984). The antimicrobial agents used were ampicillin (ABP),

cefazolin (CEZ), chloramphenicol (CP), kanamycin (KM), furazolidone (NF), sulfamonomethoxine (SA), streptomycin (SM), tetracycline (TC), trimethoprim (TMP), flumequine (FLQ), miloxacin (MLX), nalidixic acid (NA), oxolinic acid (OA), piromidic acid (PA), pipemidic acid (PPA), ciprofloxacin (CPFX), enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX).

Detection of Transferable R-Plasmid

Drug-resistant *A. hydrophila*, except for the ABP and NA resistant strains, were mixed with *Escherichia coli* K-12 RC85 met$^-$, nal^r, F$^-$ to determine transferable R-plasmids using the method of Watanabe et al. (1971).

The mixed culture was plated on bromothymol blue lactose nutrient agar containing 50 $\mu g \cdot ml^{-1}$ of NA and one of the drugs to which *A. hydrophila* was resistant. The transconjugants were purified twice on a similar medium. *E. coli* RC85 clone which received transferable R-plasmids were examined for other resistant markers.

Results

The distribution of the MIC values of 19 antimicrobial agents against 68 strains of *A. hydrophila* isolated in Thailand is shown in Figs. 1 and 2. MIC values of ABP against the strains tested ranged from 50 to > 1,000 $\mu g \cdot ml^{-1}$. The *A. hydrophila* strains were intrinsically resistant to ABP. The strains were classified into two discrete groups, which were either sensitive or resistant to CEZ, CP,

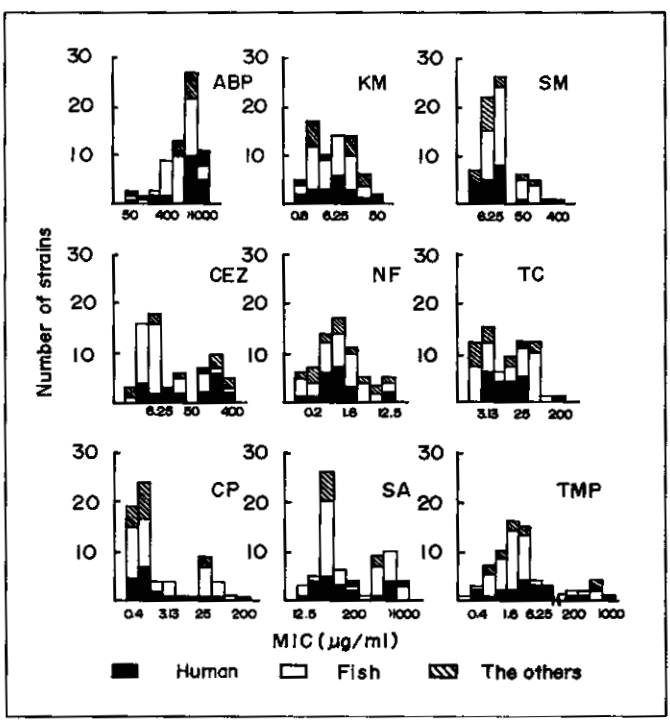

Fig. 1. Minimal inhibitory concentrations ($\mu g \cdot ml^{-1}$) of 9 antibiotics and sulfonamides for 68 strains of *Aeromonas hydrophila* (20 human isolates, 35 fish isolates, 13 other isolates, soft-shell turtle, water and sediment). Abbreviations: ABP, ampicillin; KM, kanamycin; SM, streptomycin; CEZ, cefazolin; NF, furazolidone; TC, tetracycline; CP, chloramphenicol; SA, sulfamonomethoxine; TMP, trimethoprim.

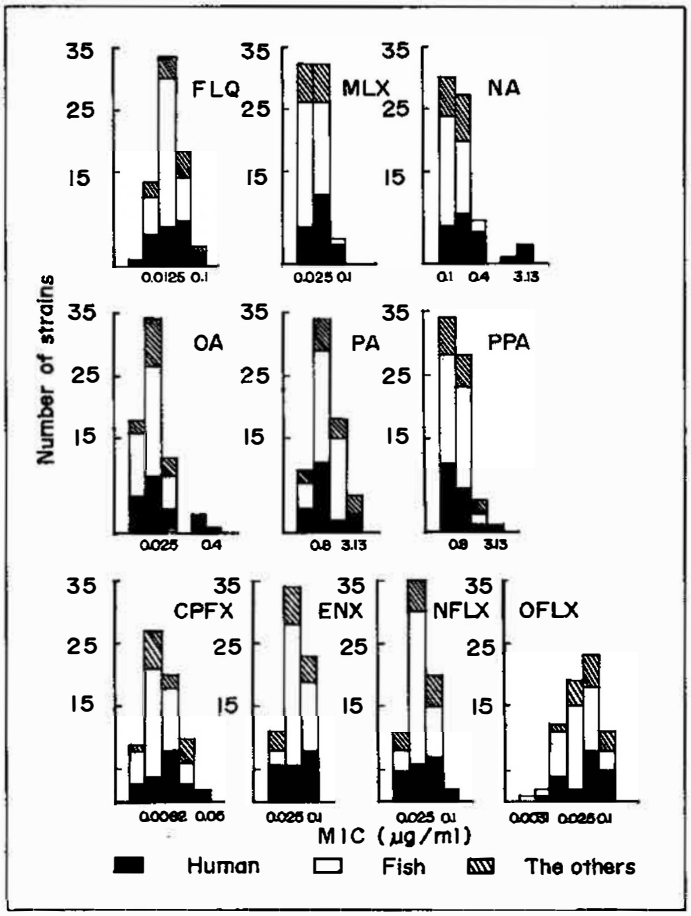

Fig. 2. Minimal inhibitory concentration ($\mu g \cdot ml^{-1}$) of 10 quinolones for 68 strains of *Aeromonas hydrophila* (Sources of isolates same as Fig. 1). Abbreviations: FLQ, flumequine; MLX, miloxacin; NA, nalidixic acid; OA, oxolinic acid; PA, piromidic acid; PPA, pipemidic acid; CPFX, ciprofloxacin; ENX, enoxacin; NFLX, norfloxacin; OFLX, ofloxacin.

SM, TC, SA, NF and TMP. Twenty-two strains were resistant to CEZ, and the remaining 46 strains were sensitive. Eighteen strains showed resistance to CP, 5 strains to NF, 34 strains to SA, 13 strains to SM, 35 strains to TC, and 9 strains to TMP. Strains which were resistant to SA and TC were isolated with high frequency (Fig.1).

All strains appeared susceptible to KM and quinolones, i.e., FLQ, MLX, NA, OA, PA, PPA, CPFX, NFLX and OFLX. Flumequine and CPFX were the most active compounds studied and their MIC values were <0.05 $\mu g \cdot ml^{-1}$ (Figs. 1 and 2).

Fourteen of the 68 strains were sensitive to all the drugs studied except for ABP. The remaining 54 strains were resistant to various combinations of drugs (Table 2). Of 54 strains, 19 were resistant to one drug, 9 to two drugs, 14 to three drugs, 8 to four drugs, 1 to five drugs, 2 to six drugs, and 1 to seven drugs.

Transferable R-plasmids were detected in 9 of 54 drug-resistant strains, all of which were isolated from snakehead. R-plasmids demonstrated from 8 strains were encoded with CP, SA and TC resistant genes. Only one strain showed resistance to TC alone (Table 2).

Table 2. Resistance markers and transferable R-plasmids of *Aeromonas hydrophila*.

Resistance marker of original strain	R⁺strains / Strains studied*			Resistance marker of R-plasmids
Sensitive strains		14	(2F, 8H, 4W)	
CEZ	0	/ 11	(3F, 4H, 4W)	
CP	0	/ 1	(1F)	
SA	0	/ 2	(1H, 1W)	
TC	1**	/ 5	(3F, 1H, 1W)	TC
CEZ, SA	0	/ 2	(1F, 1H)	
CEZ, TC	0	/ 1	(1F)	
CP, TC	0	/ 2	(1F, 1H)	
SA, SM	0	/ 1	(1H)	
SA, TC	0	/ 3	(1F, 1H, 1W)	
CEZ, SA, TC	0	/ 1	(1F)	
CP, SA, TC	7**	/ 8	(8F)	CP, SA, TC
NF, SA, TC	0	/ 1	(1F)	
SA, SM, TC	0	/ 1	(1F)	
SA, SM, TMP	0	/ 1	(1F)	
SA, TC, TMP	0	/ 2	(1F, 1H)	
CEZ, CP, SA, TC	0	/ 1	(1F)	
CEZ, SA, SM, TC	0	/ 2	(2F)	
CP, SA, SM, TMP	0	/ 1	(1F)	
NF, SA, SM, TC	0	/ 1	(1F)	
SA, SM, TC, TMP	0	/ 3	(2F, 1H)	
CEZ, CP, NF, SA, TC	0	/ 1	(1F)	
CEZ, CP, SA, SM, TC, TMP	0	/ 1	(1F)	
CEZ, CP, NF, SA, SM, TC	1**	/ 1	(1F)	CP, SA, TC
CEZ, CP, NF, SA, SM, TC, TMP	0	/ 1	(1F)	

* Number and symbols in brackets indicate the source and number of strains tested, F, fishes; H, humans; W, water or sediment.

** All strains were isolated from snakehead.

Discussion

Our results indicate that the incidence of multiple drug resistant strains of *A. hydrophila* from cultured fish are higher than those from humans in Thailand and these strains are widely distributed in fish farms. Sulfonamide and tetracycline resistant strains were commonly detected from fish farms and this is probably the result of extensive use of SA and TC in fish culture ponds.

Transferable R-plasmids were found in *A. hydrophila* isolated in Japan (Aoki et al. 1971; Akashi and Aoki 1986), Taiwan (Kou and Chung 1980) and the USA (Shotts et al. 1975). It is interesting to note that transferable R-plasmids were detected only from the drug resistant strains of *A. hydrophila* isolated from fish in Thailand. While most of the detected R-plasmids from Thailand encoded resistance to CP, TC and SA; the predominant transferable R-plasmids from Japan and Taiwan encoded resistance to TC and SA and CP, SM and SA (Aoki et al. 1971; Akashi and Aoki 1986). However, R-plasmids from the USA had resistant markers to TC, SA and CP, TC, KM and SA (Shotts et al. 1975). This suggests direct antibiotic selection pressure. The drug resistant markers of R-plasmids differed among these countries. Tetracyclines and sulfonamides were frequently used in fish cultures but not in human infections in Thailand (Saitanu 1986). Therefore, most strains were resistant to TC and SA and harbored R-plasmids.

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