Antimicrobial Susceptibility Pattern of *Edwardsiella ictaluri* Isolates from Natural Outbreaks of Bacillary Necrosis of *Pangasianodon hypophthalmus* in Vietnam

Tu Thanh Dung,¹ Freddy Haesebrouck,² Nguyen Anh Tuan,¹
Patrick Sorgeloos,³ Margo Baele,² and Annemie Decostere²

The purpose of this study was to assess the *in vitro* susceptibility of 64 Vietnamese isolates of *Edwardsiella ictaluri*, the causal agent of the infectious disease Bacillus Necrosis Pangasius in *Pangasianodon hypophthalmus*, using the agar dilution technique. All isolates originated from different farms and were collected between 2002 and 2005. None of the isolates displayed acquired resistance to amoxicillin, amoxicillin-clavulanic acid, chloramphenicol, florfenicol, gentamicin, kanamycin, neomycin, and nitrofurantoin. Acquired resistance to streptomycin was detected in 83%, to oxytetracycline in 81%, and to trimethoprim in 71% of the isolates, as indicated by a bimodal distribution of the minimal inhibitory concentrations (MICs) of these antimicrobials. The MICs of enrofloxacin displayed a monomodal distribution with tailing toward the higher MIC values, possibly indicating reduced susceptibility of a minority of isolates (3 out of the 64). For the quinolone antimicrobial agents flumequin and oxolinic acid, acquired resistance was encountered in 8% and 6% of the strains, respectively. All strains were intrinsically resistant to the polypeptide antimicrobial agent colistin. Seventy-three percent of the isolates were shown to have acquired resistance to at least three antimicrobial agents. The results of this study emphasize the strict need to control both the prophylactic and curative use of antimicrobial agents in Vietnamese aquaculture.

Introduction

TOTAL AQUACULTURE PRODUCTION in Vietnam topped 1 million tons in 2003 and is expected to reach over 2 million tons by 2010.²⁹ Mekong Delta is the main producer, being responsible for over 80% of the total Vietnamese production. The freshwater catfish *Pangasianodon hypophthalmus* is the most commonly cultured edible fish species in this region. Currently, *P. hypophthalmus* has become the second most important Vietnamese food commodity produced and exported after rice.

Of the several infectious diseases diagnosed in this fish species, Bacillary Necrosis, caused by *Edwardsiella ictaluri*, is the most frequently occurring.^{11,17} This increasingly important disease occurs in production systems of fish of all ages, although especially fingerlings and juvenile fish seem to be affected. This results in severe economic losses through decreased production, expense of treatment, and fish mortality.

Besides the Vietnamese freshwater production, the American channel catfish (*Ictalurus punctatus*) industry also suffers massively from *E. ictaluri* infections that have been termed "enteric septicemia of catfish" (ESC). ESC accounts for approximately 60% of all mortality in farmed channel

catfish, which results in approximately 50 million dollars in annual losses.³⁰ Recently, *E. ictaluri* was also identified as the cause of disease in *P. hypophthalmus* in Indonesia.⁴⁵ To treat bacterial infections, antimicrobial agents are widely used in Vietnamese aquaculture, both on a preventive and curative basis.^{11,15,33} In most of the cases, the amount of drugs to be administered merely is an estimation, leading to incorrect dosage.⁴⁰ In view of this, besides antimicrobial sensitivity monitoring being necessary for choosing effective antimicrobial agents, susceptibility testing is also crucial to monitor antimicrobial resistance development.

Hitherto, to our knowledge, only a handful of studies have investigated the antimicrobial susceptibility of *E. ictaluri*, always including American isolates. Hawke¹⁹ was the first to test the antimicrobial susceptibility of 10 *E. ictaluri* isolates. Later, Waltman and Shotts⁴² screened 118 *E. ictaluri* isolates retrieved in the United States for susceptibility to 37 antimicrobials using the disc sensitivity test. They found that the majority of isolates were susceptible to most agents active against Gram-negative bacteria, but resistance was observed against colistin and sulfonamides in more than 90% of isolates. Reger *et al.*³⁴ likewise tested the antimicrobial susceptibility of American *E. ictaluri* isolates and found full

¹College of Aquaculture and Fisheries (CAF), Cantho University, Cantho, Vietnam.

²Department of Pathology, Bacteriology, and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.

³Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Ghent, Belgium.

susceptibility to enrofloxacin, gentamicin, and doxycycline. Half a decade ago, Stock and Wiedemann³⁸ studied the antimicrobial susceptibility of 41 *E. ictaluri* strains to 71 antibiotics. All these isolates originated from American channel catfish, and hardly any acquired resistance was detected.

The aim of the present study was to investigate the antimicrobial susceptibility pattern of *E. ictaluri* isolates from *P. hypophthalmus* in Southeast Asia, more specifically Vietnam, by means of the agar dilution test.

Materials and Methods

Bacterial strains

Sixty-four *E. ictaluri* isolates from natural disease outbreaks of Bacillary Necrosis in *P. hypophthalmus* in the Mekong Delta, Vietnam, were included in this study. The isolates all originated from different farms and were collected in 2002 (37 isolates), 2003 (10 isolates), 2004 (14 isolates), and 2005 (3 isolates).

Presumptive identification was done using the API 20E commercial kit (Microbank™; PRO-LAB Diagnostics, Weston, UK).

The identity of *E. ictaluri* was confirmed by the tDNA-PCR technique.⁵ Briefly, genomic DNA was extracted by suspending one colony of a bacterial culture in 20 µl lysis buffer (0.25% sodium dodecyl sulfate, 0.05 M NaOH). After heating at 95°C for 5 minutes and centrifugation for 20 seconds at 13,000 rpm, 180 µl sterile distilled water was added and centrifugation was done at 13,000 rpm for 5 minutes. The spacers in between the tRNA genes were amplified using the primer T5B (5'-AGTCCGGTGCTCTAACCAACTGAG3') and fluorescently labeled primer T3B (5'-AGGTCGCGGGTTCG AATCC3'). PCR mixtures and cycle conditions were the same as described before.⁵ Capillary electrophoresis was carried out using the ABI Prism™ 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA). Electropherograms obtained with the Genescan software were compared to the database using in-house software.⁵ *Edwardsiella tarda* (LMG 2793T) and *E. ictaluri* (CCUG 18764) were additionally included in this assay.

Escherichia coli (ATCC 25922) was included as a reference strain in the agar dilution tests.⁹

Antibacterial agents

The following antimicrobial agents were obtained as laboratory standard powders: amoxicillin, amoxicillin + clavulanic acid, chloramphenicol, florfenicol, gentamicin, kanamycin, streptomycin, neomycin, enrofloxacin, oxolinic acid, flumequin, oxytetracycline, trimethoprim, nitrofurantoin, and colistin. These antimicrobial agents were purchased from Sigma Aldrich N.V. (Bornem, Belgium), except enrofloxacin, which was obtained from Bayer AG (Brussels, Belgium); streptomycin from Certainté (L'Alleud, Belgium); and colistin from VMD (Arendonk, Belgium). They were dissolved in appropriate solvents to make stock solutions and then further diluted in sterile distilled water according to the methods recommended by the Clinical and Laboratory Standards Institute (CLSI M49-A).⁹

Susceptibility testing

Minimal inhibitory concentration (MIC) tests were carried out on Mueller-Hinton II agar (Becton Dickinson, Cockeys-

ville, MD) containing doubling dilutions of the antimicrobials. Final concentrations of 0.12–128 µg/ml were tested for all antibacterial agents, and antibiotic-free agar plates were included as a control for normal growth.

All the *Edwardsiella* isolates were inoculated on Columbia blood agar (Difco, Wesel, Germany) and incubated for 48 hours at 26°C. The *E. coli* reference strain ATCC 25922 was likewise inoculated on Columbia blood agar, but incubation occurred for 24 hours at 37°C. Inocula were prepared by suspending overnight cultures in phosphate-buffered saline (PBS) to a density of 0.5 on the McFarland scale. Consequently, 1/10 dilutions in PBS were prepared. Approximately 1×10⁵ colony forming units of the strains were then inoculated on the antibiotic-containing plates and on antibiotic-free control plates by means of a Denley multi-point inoculator (Mast, Sussex, UK), after which plates were incubated aerobically at 26°C. For *Edwardsiella* isolates, MICs were recorded after 48 hours and for the *E. coli* reference strain after both 24 and 48 hours' incubation. The MIC was defined as the lowest concentration of the antimicrobial agent with no visible bacterial growth. For interpretation of MIC results, the microbiological criterion (epidemiological or wild-type cut-off value) was used.³⁹

Results

MIC endpoints of the *E. coli* reference strain did not differ when plates were incubated during 24 or 48 hours and for the antimicrobial agents included in Clinical and Laboratory Standards Institute document M49-A; they fell within acceptable quality ranges.⁹

An overview of the MIC values for the different *E. ictaluri* isolates is shown in Table 1. The MIC₅₀ and MIC₉₀ values and percentages of isolates considered to have acquired resistance are likewise presented.

For the β-lactam antimicrobial agents amoxicillin and the combination of amoxicillin-clavulanic acid, as well as for chloramphenicol, florfenicol, nitrofurantoin, and the aminoglycoside antibiotics gentamicin, kanamycin, and neomycin, a monomodal distribution of MICs was noted, indicating absence of acquired resistance. In contrast, for streptomycin, oxolinic acid, flumequin, oxytetracycline, and trimethoprim, the MICs showed a bimodal distribution. According to the microbiological criterion, isolates in the higher range of MICs should be considered to have acquired resistance.³⁹ For enrofloxacin, tailing toward the higher MIC values was observed, possibly indicating reduced susceptibility in three isolates.

All *E. ictaluri* isolates were intrinsically resistant to the polypeptide antimicrobial agent colistin with MIC values equal to or above 64 µg/ml.

Resistance phenotypes of the *E. ictaluri* isolates are presented in Table 2.

Discussion

Different criteria may be used for the interpretation of MIC results. For several terrestrial animals, interpretive criteria or breakpoints have been established by the Clinical and Laboratory Standards Institute (CLSI document M31-A3) so that the results of the tests can be interpreted as susceptible, intermediate, or resistant and reported as such to veterinarians.¹⁰ For selection of these breakpoints, a range of para-

TABLE 1. DISTRIBUTION OF MINIMAL INHIBITORY CONCENTRATION (MIC) OF VARIOUS ANTIMICROBIAL AGENTS ON 64 *EDWARDSIELLA ICTALURI* ISOLATES FROM *PANGASIANODON HYPOPHthalmus* IN VIETNAM

Antimicrobial agent	Number of isolates with MIC ($\mu\text{g/ml}$) of												MIC ₅₀	MIC ₉₀	% Resistance ^a
	≤ 0.12	0.25	0.5	1	2	4	8	16	32	64	≥ 128				
Amoxicillin	3	19	42										0.5	0.5	0
Amoxicillin + clavulanic acid	42	22											0.12	0.25	0
Chloramphenicol	13	28	23										0.25	0.5	0
Florfenicol	31	33											0.25	0.25	0
Gentamicin				15	34	15							2	4	0
Kanamycin					18	39	7						4	8	0
Streptomycin						2	9		1	1	51	≥ 128	≥ 128		82.8
Neomycin					2	52	10					4	8		0
Enrofloxacin	9	27	11	14	3							0.25	1		4.7
Oxolinic Acid				1	9	21	27	2		4		8	8		6.3
Flumequin		1		3	9	46			2	3		4	4		7.8
Oxytetracycline	2		8	1	1			3	26	23		32	64		81.3
Trimethoprim	9				8					13	34	≥ 128	≥ 128		73.4
Nitrofurantoin		8	8	5	34	9						2	4		0
Colistin										3	61	≥ 128	≥ 128		Intrinsically resistant

^a*E. ictaluri* isolates considered to have acquired resistance according to the microbiological criterion are represented in bold.

meters are used, including results of *in vitro* susceptibility tests on a large number of isolates and pharmacokinetic/pharmacodynamic data analyzing susceptibility data in relation to pharmacokinetics of the antimicrobial agent from normal dosing regimens. Other parameters used for establishing interpretive criteria of MIC results are *in vitro* resistance markers, both phenotypic and genotypic, as well as clinical and bacteriological outcome data from clinical studies.³⁹ For aquatic pathogens, however, most of these data are lacking, and Clinical and Laboratory Standards Institute breakpoints are not available.⁹ Therefore, in the present study, the microbiological criterion was used for interpretation of MIC results. This parameter allows distinguishing wild-type populations of bacteria from those with acquired resistance. Using this criterion, interpretation of MIC results was straightforward for most antimicrobial agents tested here, because MIC distributions were either monomodal, indicating absence of acquired resistance, or bimodal, indicating acquired resistance in isolates in the higher range of MIC values. Only for enrofloxacin, MICs rather showed an extended

frequency distribution range, and the division between isolates with or without acquired resistance was more difficult to establish.

The microbiological criterion refers to direct *in vitro* interactions between the antimicrobial agents and the *E. ictaluri* isolates and does not necessarily predict how the patient will respond to therapy. However, for most antimicrobial agents tested here, MIC values were at least 10 times higher for isolates with acquired resistance. The likelihood that fish infected with these isolates will respond well to treatment with this antimicrobial agent should be considered to be low.

Chloramphenicol has been strictly prohibited for animal use in most countries, including Vietnam, because of its severe toxicity and causing depression of the bone marrow.^{29,31} Its fluorinated derivative, florfenicol, has instead become available and rapidly became popular in several animal industries, including aquaculture.^{14,18,28,44} All *E. ictaluri* isolates included in the present study were highly susceptible to florfenicol, with an MIC $\leq 0.25 \mu\text{g/ml}$. McGinnis likewise tested *E. ictaluri* isolates from American channel catfish and found full susceptibility.²⁶ In contrast, Ho *et al.*²⁰ isolated *E. tarda*, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Vibrio cholerae*, and *Salmonella* spp. isolates from aquatic animals in Taiwan that were less susceptible to florfenicol. This finding emphasizes the need to be vigilant and control the use of florfenicol in the sense that it should not be used indiscriminately.

In the present study, acquired resistance to the aminoglycoside antibiotics gentamicin, kanamycin, and neomycin was not detected in the *E. ictaluri* isolates. In contrast, the MIC values for streptomycin showed a bimodal distribution, with 83% of the isolates displaying an MIC value in the higher range and thus having acquired resistance. Previous studies showed full susceptibility of *E. ictaluri* and *E. tarda* to streptomycin,^{19,32,38,42} rendering this study the first to report high levels of resistance to this antibiotic.

All isolates in our study clearly displayed intrinsic resistance to the polypeptide antimicrobial agent colistin. This

TABLE 2. RESISTANCE PHENOTYPES OF 64 *EDWARDSIELLA ICTALURI* ISOLATES FROM *PANGASIANODON HYPOPHthalmus* IN VIETNAM

Resistance phenotype	Number (%) of isolates
SM	4 (6.3)
OTC	2 (3.1)
SM + OTC	2 (3.1)
OTC + TMP	1 (1.6)
SM + OTC + TMP	42 (65.6)
SM + OTC + TMP + FM	1 (1.6)
SM + OTC + OXO + FM	1 (1.6)
SM + OTC + TMP + OXO + FM + ENR	3 (4.7)
No acquired resistance	8 (12.5)

SM, streptomycin; OTC, oxytetracycline; TMP, trimethoprim; FM, flumequin; OXO, oxolinic acid; ENR, enrofloxacin.

finding correlates with the results from a previous study performed by Muyembe *et al.*,³² who found that all 35 human isolates of *E. tarda* were resistant to colistin. Waltman and Shotts⁴² screened 118 *E. ictaluri* isolates retrieved in the United States for susceptibility to 37 antimicrobials using the disc sensitivity test. The study found that the majority of isolates were susceptible to most agents active against Gram-negative bacteria, but resistance was observed against colistin in more than 90% of isolates. The findings suggested that colistin could be used as an additive to generate a selective medium for the isolation of *Edwardsiella* species, including *E. ictaluri*.

In the present study, three strains displayed reduced sensitivity to enrofloxacin, for which an MIC value of 2 µg/ml was noted. Fluoroquinolones inhibit the activity of DNA gyrase, and in most bacterial species resistance is due to mutations in the gyrase or topoisomerase genes.^{6,37,43} In *Enterobacteriaceae* resistance to quinolones is most commonly acquired in two steps. One mutation in the *gyrA* genes mediates full resistance to first-generation quinolones, such as nalidixic acid and flumequin, and reduced susceptibility to other fluoroquinolones. A second mutation in either *gyrA* or *gyrB* genes mediates full resistance to fluoroquinolones.^{16,41} In the present study, the three isolates with reduced sensitivity to enrofloxacin indeed displayed resistance toward flumequin. Further research will be needed to elucidate the mechanisms of enrofloxacin resistance found in these isolates.

For the antimicrobial agents flumequin and oxolinic acid, clear bimodal distributions of MICs were evident and acquired resistance was encountered in 8% and 6% of the strains, respectively. As far as we know, this is the first report of resistance toward these antimicrobial agents in *E. ictaluri*. Stock and Wiedemann³⁸ did not find any resistance against quinolone agents among the *Edwardsiella* species tested, including *E. ictaluri*.⁴² Presently, only oxolinic acid is allowed for use in Vietnamese aquaculture. The newer quinolones such as enrofloxacin are strictly banned.³¹ Presently, the European Medicines Agency¹⁶ reported that (fluoro)quinolones are among the most important antimicrobial agents for treatment of severe and invasive infections in human and animals. The use of fluoroquinolones in aquaculture should, therefore, always be carefully considered and controlled by the food and drug national authority.

In this study, the distribution of MIC values of oxytetracycline was clearly bimodal, with the majority of strains falling in the higher range (≥ 16 µg/ml), indicating acquired resistance in 81% of the isolates. The high percentage of oxytetracycline resistance encountered in this study most likely is related to the wide and sometimes improper use of this antimicrobial agent in aquaculture.^{1,2,12,13,35,40} Several authors have done research on the distribution of *tet* genes in tetracycline-resistant fish pathogens, focusing on *E. tarda*.^{4,23,24,36} Unfortunately, information on genotypic susceptibility of *E. ictaluri* is frequently lacking and hence hitherto poorly understood. In view of the high resistance percentages noted, this certainly merits further investigation.

Similarly to oxytetracycline, 73% of the isolates had acquired resistance to trimethoprim. According to Hawke¹⁹ and Waltman and Shotts,⁴² oxytetracycline and trimethoprim are used for treatment of ESC caused by *E. ictaluri* in industrial catfish cultured in the United States. Likewise, oxytetracycline and trimethoprim were denoted as being the

most commonly used antimicrobial agents in fish and shrimp in Vietnam.^{15,25,33,42}

The present study pointed out that 73.4% of the isolates had acquired resistance to at least three antimicrobial agents. These isolates, when causing disease, may be especially difficult to control. This phenomenon may even become more cumbersome when one considers the fact that multiple resistance transfer by plasmids does occur and even is considered a problem of major concern in aquatic antimicrobial therapy.³ Plasmid-mediated resistance to chloramphenicol, trimethoprim, sulphonamides, and tetracyclines has indeed been identified in fish pathogens.²⁷ Plasmids transferring resistance to as many as five antimicrobials have been identified from marine and freshwater fish pathogens—for example, *Vibrio anguillarum*, *Vibrio salmonicida*, *Aeromonas salmonicida*, *A. hydrophila*, *E. tarda*, and *Yersinia ruckeri*.^{4,10,11}

The high number of isolates simultaneously displaying resistance to streptomycin, oxytetracycline, and trimethoprim may suggest the presence of an epidemic clone in the area from which our isolates were obtained. Alternatively, it may indicate a high prevalence of plasmids carrying resistance genes to these three antimicrobial agents in non-clonal isolates.

Several researchers have pointed toward hygienic shortcomings in fish-rearing methods. This, in combination with increased fish population densities, crowding of farming sites, and lack of sanitation barriers, has facilitated the rapid spread of infectious agents leading to the prophylactic use of antimicrobial agents, often with the misplaced goal of forestalling these sanitary shortcomings.^{8,37,40} Overuse of antimicrobial agents favors spread of antimicrobial-resistant bacteria in the aquaculture environment and the emergence of antimicrobial resistance in fish pathogens as demonstrated in this study. This may result in the transfer of these resistance determinants to bacteria of terrestrial animals and human pathogens, and in alterations of the bacterial microbiota both in sediments and in the water column.^{7,21,22,25}

Further studies are necessary to elucidate the genetic mechanisms of the encountered resistance. In the meantime, there is a strict need to control both the prophylactic and curative use of antimicrobial agents in Vietnamese aquaculture.

Acknowledgments

The authors thank Ms. Nguyen Thi Nhu Ngoc, Mr. Nguyen Quoc Thinh, and Mrs. Dang Thuy Mai Thy for their tremendous help in collecting the isolates. We are also grateful to Mrs. Arlette Van de Kerckhove and Mr. Gunter Massaer for their excellent technical assistance. Financial support from DFID (Project No. R8093) is gratefully acknowledged.

Disclosure Statement

No competing financial interests exist.

References

1. Akinbowale, O.L., H. Peng, P. Grant, and M.D. Barton. 2007. Antibiotic and heavy metal resistance in motile *Aeromonads* and *Pseudomonads* from rainbow trout (*Oncorhynchus*

- mykiss*) farms in Australia. *Int. J. Antimicrob. Agents* **30**: 177–182.
2. Alderman, D.J., and T.S. Hastings. 1998. Antibiotic use in aquaculture: development of antibiotic resistance-potential for consumer health risks. *Int. J. Food Sci. Tech.* **33**:139–155.
 3. Aoki, T. 1988. Drug resistance plasmids from fish pathogens. *Microbiol. Sci.* **5**:219–223.
 4. Aoki, T., and A. Takahashi. 1987. Class D tetracycline resistance determinants of R plasmids from the fish pathogens *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Pasteurella piscicida*. *Antimicrob. Agents Chemother.* **31**:1278–1280.
 5. Baele, M., P. Baele, M. Vaneechoutte, V. Storms, P. Butaye, L.A. Devriese, G. Verschraegen, M. Gillis, and F. Haesebrouck. 2000. Application of tDNA-PCR for the identification of *Enterococcus* species. *J. Clin. Microbiol.* **38**:4201–4207.
 6. Barnes, A.C., C.S. Lewin, T.S. Hastings, and S.G.B. Amyes. 1991. Fluoroquinolones display rapid bactericidal activity and low mutation frequencies against *Aeromonas salmonicida*. *J. Fish Dis.* **14**:661–667.
 7. Cabello, F.C. 2004. Antibiotics and aquaculture. An analysis of their potential impact upon the environment, human and animal health in Chile. *Fundacion Terram. Analisis de Politicas Publicas.* **17**:11–16.
 8. Cabello, F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for environment. *Environ. Microbiol.* **8**:1137–1144.
 9. Clinical and Laboratory Standards Institute (CLSI). 2006. Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals; informational supplement, M49-A, Clinical and Laboratory Standards Institute, Wayne, NJ.
 10. Clinical and Laboratory Standards Institute (CLSI). 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, third edition, M31-A3, Clinical and Laboratory Standards Institute, Wayne, NJ.
 11. Crumlish, M., T.T. Dung, J.F. Turnbull, N.T.N. Ngoc, and H.W. Ferguson. 2002. Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. *J. Fish Dis.* **25**:733–736.
 12. DePaola, A., P.A. Flynn, R.M. McPhearson, and S.W. Levy. 1988. Phenotypic and genotypic characterization of tetracycline- and oxytetracycline-resistant *Aeromonas hydrophila* from cultured channel catfish (*Ictalurus punctatus*) and their environments. *Appl. Environ. Microbiol.* **54**:1861–1863.
 13. DePaola, A., J.T. Peeler, and G.E. Rodrick. 1995. Effect of oxytetracycline-mediated feed on antibiotic resistance of gram-negative bacteria in catfish ponds. *Appl. Environ. Microbiol.* **61**:2335–2340.
 14. Dowling, P.M. 2006. Chloramphenicol, thiamphenicol and florfenicol. In S. Giguère, J.F. Prescott, D. Baggot, R.D. Walker, and P.M. Dowling (eds.), *Antimicrobial therapy in veterinary medicine*. Blackwell Publishing Ltd, Oxford, pp. 241–248.
 15. Dung, T.T., Z. Galina, D.T.H. Oanh, Z. Jeney, and N.A. Tuan. 1997. Results of the baseline survey on fish health management in freshwater aquaculture of the Mekong Delta, Vietnam. *WEST-EAST-SOUTH (WES) Newsletter* No. 6.
 16. European Medicines Agency. Veterinary Medicines and Inspections (EMA). 2006. Reflection paper on the use of fluoroquinolones in food-producing animals in the European Union: development of resistance and impact on human and animal health. EMA/CVMP/SAGAM/184651/2005-consultation, London, UK, 23 pp. Available online at www.emea.europa.eu/pdfs/vet/srwp/18465105en.pdf.
 17. Ferguson, H.W., J.F. Turnbull, A. Shinn, K. Thompson, T.T. Dung, and M. Crumlish. 2001. Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. *J. Fish Dis.* **24**:509–513.
 18. Gaunt, P., R. Endris, L. Khoo, T. Leard, S. Jack, T. Santucci, T. Katz, S.V. Radecki, and R. Simmons. 2003. Preliminary assessment of the tolerance and efficacy of florfenicol against *E. ictaluri* administered in feed to channel catfish. *J. Aquat. Anim. Health* **15**:239–247.
 19. Hawke, J. 1979. A bacterium associated with disease of pond cultured catfish, *Ictalurus punctatus*. *J. Fish. Res. Board Can.* **36**:508–1512.
 20. Ho, S.P., T.Y. Hsu, M.H. Chen, and W.S. Wang. 2000. Antibacterial effect of chloramphenicol, thiamphenicol and florfenicol against aquatic animal bacteria. *J. Vet. Med. Sci.* **62**:479–485.
 21. Holmström, K., S. Graslund, A. Wahlstrom, S. Pongshompoo, B. Bengtsson, and N. Kautsky. 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. *Int. J. Food Sci. Tech.* **38**:255–266.
 22. Huys, G., D. Gevers, R. Temmerman, M. Cnockaert, R. Denys, G. Rhodes, R. Pichup, P. McGann, M. Hiney, P. Smith, and J. Swings. 2001. Comparison of the antimicrobial tolerance of oxytetracycline-resistant heterotrophic bacteria isolated from hospital sewage and freshwater fishfarm water in Belgium. *Syst. Appl. Microbiol.* **24**:122–130.
 23. Jun, L.J., J.B. Jeong, M.D. Huh, J.K. Chung, D.L. Choi, C.H. Lee, and H.D. Jeong. 2004. Detection of tetracycline-resistance determinants by multiplex polymerase chain reaction in *Edwardsiella tarda* isolated from fish farms in Korea. *Aquaculture* **240**:89–100.
 24. Kim, S.R., L. Nonaka, and S. Suzuki. 2004. Occurrence of tetracycline resistance genes *tet(M)* and *tet(S)* in bacteria from marine aquaculture sites. *FEMS Microbiol. Lett.* **237**: 147–156.
 25. Le, T.X., Y. Munekage, and S.I. Kato. 2005. Antibiotic resistance in bacteria from shrimp farming in mangrove areas. *Sci. Total Environ.* **349**:95–105.
 26. McGinnis, A., P. Gaunt, T. Santucci, R. Simmons, and R. Endris. 2003. *In vitro* evaluation of the susceptibility of *Edwardsiella ictaluri*, etiological agent of enteric septicemia in channel catfish, *Ictalurus punctatus* (Rafinesque), to florfenicol. *J. Vet. Diagn. Invest.* **15**:576–579.
 27. McPhearson, R.M., A. DePaola, S.R. Zywno, M.L. Motes, and A.M. Guarino. 1991. Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. *Aquaculture* **99**:203–211.
 28. Michel, C., B. Kerouault, and C. Martin. 2003. Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. *J. Appl. Microbiol.* **95**:1008–1015.
 29. Ministry of Fisheries. 2005. Vietnam Fisheries Sector Programme Support, Phase II 2006–2010. Project ref. 104. Vietnam:803–200.
 30. Mitchell, A.J. 1997. Fish disease summaries for the southeastern United States from 1976–1995. *Aquacult. Mag.* **23**:87–93.
 31. MoFi. 2004. Ministry of Fisheries in Vietnam. Department of Legislation—Fisheries Law Project. Available at <http://www.mofi.gov.vn/law/default.aspx?tabid=288&ID=578>. (Online.)

32. **Muyembe, T., J. Vandepitte, and J. Desmyter.** 1973. Natural colistin resistance in *Edwardsiella tarda*. *Antimicrob. Agents Chemother.* **4**:521–524.
33. **Phuong, N.T., D.T.H. Oanh, T.T. Dung, and L.X. Sinh.** 2005. Bacterial resistance to antimicrobials use in shrimps and fish farms in the Mekong Delta, Vietnam. *In* Proceedings of the international workshop on antibiotic resistance in Asian aquaculture environments. Chiang Mai, Thailand, pp. 2–17. ISBN 88-901344-3-7. Available online at <http://www.medinfo.dist.unige.it/asiaregist/workshop.htm>.
34. **Reger, P.J., D.F. Mockler, and M.A. Miller.** 1993. Comparison of antimicrobial susceptibility, beta-lactamase production, plasmid analysis and serum bactericidal activity in *Edwardsiella tarda*, *E. ictaluri* and *E. hoshinae*. *J. Med. Microbiol.* **39**:273–281.
35. **Reimschuessel, R., and R.A. Miller.** 2006. Antimicrobial drug use in aquaculture. *In* S. Giguère, J.F. Prescott, D. Baggot, R.D. Walker, and P.M. Dowling (eds.), *Antimicrobial therapy in veterinary medicine*. Blackwell Publishing Ltd, Oxford, pp. 593–606.
36. **Roberts, M.C.** 2005. MiniReview: update on acquired tetracycline resistance. *FEMS Microbiol. Lett.* **245**:195–203.
37. **Sorum, H.** 2006. Antimicrobial drug resistance in fish pathogens. *In* F.M. Aarestrup (ed.), *Antimicrobial resistance in bacteria of animal origin*. American Society for Microbiology Press, Washington, DC, pp. 213–238.
38. **Stock, I., and B. Wiedemann.** 2001. Natural antimicrobial susceptibility of *Edwardsiella tarda*, *E. ictaluri* and *E. hoshinae*. *Antimicrob. Agents Chemother.* **45**:2245–2255.
39. **Turnidge, J., and D.L. Paterson.** 2007. Setting and revising antibacterial susceptibility breakpoints. *Clin. Microbiol. Rev.* **20**:391–408.
40. **Van, P.T.** 2005. Current status of aquaculture veterinary drugs usage for aquaculture in Vietnam. *In* Proceedings of the international workshop on antibiotic resistance in Asian aquaculture environments. Chiang Mai, Thailand, pp. 1–3. ISBN 88-901344-3-7. Available online at <http://www.medinfo.dist.unige.it/asiaregist/workshop.htm>.
41. **Walker, R.D., and P.M. Dowling.** 2006. Fluoroquinolones. *In* S. Giguère, J.F. Prescott, D. Baggot, R.D. Walker, and P.M. Dowling (eds.), *Antimicrobial therapy in veterinary medicine*. Blackwell Publishing Ltd, Oxford, pp. 263–284.
42. **Waltman, W.D., and E.B. Shotts.** 1986. Antimicrobial susceptibility of *Edwardsiella ictaluri*. *J. Wildl. Dis.* **22**:173–177.
43. **Wang, H., J.L. Dzink-Fox, M. Chen, and S.B. Levy.** 2001. Genetic characterization of highly fluoroquinolone-resistant clinical *Escherichia coli* strains from China: role of *acrR* mutations. *Antimicrob. Agents Chemother.* **45**:1515–1521.
44. **Wrzesinski, C., L. Crouch, P. Gaunt, D. Holifield, N. Bertrand, and R. Endris.** 2005. Florfenicol residue depletion in channel catfish, *Ictalurus punctatus* (Rafinesque). *Aquaculture* **253**:309–316.
45. **Yuasa, K., E.B. Kholidin, N. Panigoro, and K. Hatai.** 2003. First isolation of *Edwardsiella ictaluri* from cultured striped catfish *Pangasius hypophthalmus* in Indonesia. *Fish Pathol.* **38**:181–183.

Address reprint requests to:

Prof. Dr. Freddy Haesebrouck

Department of Pathology, Bacteriology, and Avian Diseases

Ghent University

Salisburylaan 133

9820 Merelbeke

Belgium

E-mail: freddy.haesebrouck@ugent.be